THE EXTENT OF VARIATIONS IN THE BLOOD PICTURE OF NORMAL DOGS AND THE DEVIATIONS ENCOUNTERED IN SOME CANINE DISEASES

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S U M M A R Y

This work is divided into two sections. The first deals with variations in the blood picture of normal dogs under a variety of conditions and the second with variations in two diseases.

In the first section over three hundred dogs have been examined. These dogs came from several different sources and were of varying ages and breeds.

The results obtained indicate that the blood picture is influenced to a considerable extent by the age of the dogs and by the environment in which it lives. The red cell counts are mainly affected by age and the total and differential white cell counts by environment.

Pregnancy causes variations in both red and white cell counts and these changes are most marked towards the end of gestation. Oestrus periods cause slight variations in the blood picture but false pregnancy does not. It is possible, in certain circumstances, to diagnose pregnancy by means of a blood picture.

Breed does not influence the blood picture in dogs other than greyhounds.

The blood picture is not altered appreciably by sex differences, or by exercise, feeding, travelling or work.

Twelve different estimations have been investigated and a standard normal blood picture has been drawn up for dogs including all these estimations. The morphological characteristics of the various /

various types of blood cells has been discussed and special staining techniques investigated with regard to eosinophils.

The wide ranges of variations, given by other authors, for red and white cell estimations, have been shown to occur and the causes of these variations have been precisely defined.

In the second section of this work two clinical conditions, pyometra and mammary tumours, have been investigated. Dogs suffering from pyometra have been shown to have alterations in their blood pictures and these changes have been correlated with the clinical signs and the condition of the uterus.

No significant changes in the blood picture was observed in animals with mammary tumours unless necrosis was present.

GENERAL INTRODUCTION

The work to be embodied in this thesis can be divided into two main sections. The first and larger section deals with the peripheral blood picture of normal dogs under a variety of different conditions, while the second section is concerned with the peripheral blood picture of dogs suffering from various pathological conditions.

These two sections are inter-related in that the results obtained from the normal dogs will be used as a guide to assess the significance of changes observed in the blood of abnormal dogs.

A considerable number of estimations, which will be described in detail later, were carried out on the blood obtained from normal dogs. The dogs were of various ages and sexes and living in different environments. This latter aspect is one which has been neglected in previous work. In addition, samples were examined from normal dogs undergoing forms of physiological stress including pregnancy, oestrus periods, exercise and feeding.

In the second section blood samples obtained from dogs suffering with pyometra or mammary tumours were examined.

The whole of this work has been undertaken from a clinical standpoint, the object being to find variations in the blood of normal and abnormal dogs which are of clinical significance.

This thesis is in two volumes. The first contains the text /

text and some tables. The second is composed entirely of tables which are arranged in the same order as the text. The text can be read and the applicable results seen at the same time by using both volumes together.

MATERIALS AND METHODS OF EXAMINATION

COLLECTION OF BLOOD

Venous blood was withdrawn from the cephalic vein without the use of a syringe.

Size 8/10 m.m., 15 m.m. hypodermic needles (Arnold) were used for this. They were all siliconed before use with M.441 Silicone (I.C.I.).

The right fore leg was usually selected. The hair above the vein was clipped away with curved scissors, and the dog was restrained by an assistant who held the leg in extension. The clipped area was swabbed with alcohol. The assistant raised the vein by pressure of his thumb on the leg in front of the elbow joint. The skin was stretched over the vein and the thumb of the operator's left hand used to steady the vein. The needle was then inserted through the skin directly into the vein. If the vein was not entered first time, as occasionally happened, the needle was pushed into the vein without withdrawing it through the skin. The needle was then adjusted until a steady flow of blood came from it (usually as a series of drops).

The blood was allowed to drip directly into the bottles used for its collection, without running down the hair of the leg. Approximately 2 c.c. were collected. A sterilised wire loop was then used to collect a drop of blood from the needle and this drop was placed on two slides, so that blood films could be prepared.

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The assistant released his pressure on the vein, the needle was withdrawn, and pressure applied to the leg with a pad of cotton wool to stop any bleeding.

Corks were replaced in the bottles immediately and the blood mixed with the anticoagulent (see overleaf) by rotating the bottles between the palms of the hands. As soon as this was done the blood films were prepared.

All blood was collected by this method except for the day-old puppies. In these cases blood was withdrawn by cardiac puncture using a syringe.

PREPARATION OF BLOOD FILMS

The small spot of blood obtained direct from the vein, and placed on the slides, was used to prepare the blood films.

The slides used were chemically clean, grease free slides. They were stored, after cleaning, in alcohol, and were dried and cleansed prior to use. A "spreader" was used to make the blood films. This consisted of a thick glass slide with one end narrower than the other. The narrow end had an absolutely smooth leading edge used for spreading. The spreader was placed just in front of the drop of blood and then moved back until the two came in contact. The blood then spread along the leading edge of the spreader, which was held at an angle of approximately 30 degrees and smoothly but quickly pushed forward. By this action the blood was drawn out into a thin even smear.

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The two films made in this way were dried quickly by waving them in the air. Unless this was done cellular distortion occurred. This could be seen in films prepared on humid days when they did not dry out as quickly as usual.

A good film was thin, light brown in colour, and evenly spread. The edges of the film did not come to the edge of the slide and the end of the smear did not have "tails" present. Such tails are caused by a rough edge on the spreader. Two films were made from each dog.

ANTICOAGULENTS

Two anticoagulents were used in this work.

Sequestrene - the disodium salt of ethylene - diaminetetra - acetic acid (E.D.T.A.) was used as a 10% aqueous solution at the rate of 0.01 ml. per ml. of blood.

A mixture of ammonium oxalate (1.2 grm.) and potassium oxalate (0.8 grm.) was also used, Heller & Paul (1934). This was made up to 100 mls. with distilled water and used at the rate of 0.2 mls. per 2 mls. of blood.

Literature on Anticoagulents

Several authors have described the excellent anti-coagulent properties of E.D.T.A. especially with regard to its preservation of cellular elements. Schmidt, Hane and Gomez (1953) and Wittgenstein (1953) compared it with other anti-coagulents and found it excellent with regard to packed cell volumes, total white, and differential white counts. Proescher (1951) also had good results with ethylene bis-iminodiacetic acid.

Hadley /

Hadley & Larson (1953) found platelet counts higher using E.D.T.A. when compared with oxalate anticoagulents. Hadley & Weiss (1955) also had similar results.

It was known, however, that sequestrene used alone as an anticoagulent gave inaccurate sedimentation rate results. The reference for this was not known at the time this work commenced, and so it was decided that Heller & Paul mixture, a well tried anticoagulent, should be used for all red cell estimations including sedimentation rates.

The work of Sacker, Saunders, Page & Goodfellow (1959) confirmed the belief concerning sedimentation rates. They found that Dilithium Sequestrene gave erroneous sedimentation rates, especially if the blood was allowed to stand for some time. Denson (1959) also had similar findings using sequestrene alone. However he obtained good sedimentation results if sodium citrate was added to the sequestrene before use, and Melville & Rifkind (1959) had identical findings.

As this work on sodium citrate - sequestrene mixtures was not located until the work for this thesis was well under way it was decided to carry on using ammonium and potassium oxalate rather than change over to another anticoagulent for the red cell estimations.

Sequestrene was used in siliconed bottles. Platelet counts, eosinophil counts and total white counts were done on blood from these bottles. Red cell counts, packed cell volume, haemoglobin /

haemoglobin, specific gravity, reticulocyte counts, red cell fragility and sedimentation rates were performed on blood from non-siliconed bottles using oxalates as the anticoagulent.

Both anticoagulents were introduced into the blood collection bottles in the liquid state and then dried in an oven at 37°C before use.

CONTAINERS FOR BLOOD COLLECTION AND METHOD OF SILICONIZING

Small glass bottles holding up to 4 c.c. were used. Half of these bottles were thoroughly cleaned but not siliconed, ammonium and potassium oxalate was intfoduced as the anticoagulent.

The remaining bottles were siliconed. They were re-siliconed after being used. Sequestrene was used as the anti-coagulent. The bottles were stoppered with siliconed rubber bungs.

Siliconing was done using silicone M.441 (I.C.I.) and was performed in the following manner:-

The bottles were washed in detergent until clean. After this they were washed in tap water, then distilled water, and dried in an oven at 40°C. When they were dry, silicone M.441 was poured into each bottle, allowed to stand for a few seconds, and was then poured out again. The bottles were washed in cold water for several minutes, distilled water for a minute and then rinsed with 2% ammonia to neutralize any remaining silicone. The bottles were finally washed in distilled water and dried in the oven. Anticoagulent was added as soon as the bottles were dry.

All /

All needles were siliconized in a similar manner.

The first part of this process was performed in the fume cupboard and rubber gloves were worn, as this type of silicone has pungent acrid fumes and is highly corrosive. This method of siliconizing proved to be very efficient and the results excellent.

TRANSPORTATION OF COLLECTED BLOOD SAMPLES

It was necessary in this work to travel some distance to bleed dogs, most of which were located outside the College. In order to transport blood safely a special box was made holding bottles and slides for up to six dogs. The bottles were held upright by means of spring clips and the slides fitted into slots specially made for this purpose. A tray under the lid of the box contained room for needles, spreaders, cotton wool, bottles of alcohol and wire loops.

This box allowed for easy transportation of bottles and slides without fear of the blood spilling or deterioration of the blood films during transit.

WEIGHING DOGS

A portable spring balance weighing up to 200 lbs. was used. One or two roller towels (depending on the size of the dog) were used as a sling. The towel passed round each side of the neck, between the fore limbs and was then drawn up either side of the dog's chest, thus making a loop above the dog's back into which /

which the hook of the balance fitted. The dog was lifted off the ground and the weight read from the scale on the balance. The weight of the towel was deducted to give the dog's true weight.

METHOD USED FOR HAEMOGLOBIN ESTIMATION

The alkaline haematin method, Clegg & King (1942) was used throughout the work.

The haemoglobin was converted with alkali to alkaline haematin, and the colour thus produced was compared in an EEL colorimeter with an artificial haemoglobin standard.

Method

4.95 mls. of 0.1 N. sodium hydroxide was placed in a test tube. Blood was drawn up to exactly the 50 cu.m.m. mark in a Sahli pipette. The outside of the pipette was wiped clean with cotton wool and the contents blown into the sodium hydroxide. Any traces of blood were removed by alternately sucking sodium hydroxide in and out of the pipette. The contents of the tube were then well mixed. 4.95 mls. Gibson & Harrison artificial haemoglobin standard (B.D.H.) was placed in a second tube. These two tubes were placed in boiling water for exactly 4 minutes. In cases where foetal haemoglobin was present in the blood, boiling was continued for 15 minutes, care being taken to prevent excess evaporation. The tubes were removed and rapidly cooled. As soon as they were cold the values were read on an EEL colorimeter using a No. 404 green filter, the machine having been first zeroed with distilled water.

The haemoglobin standard was equal to exactly 16 grms. haemoglobin per 100 mls. after boiling. The following calculation was employed to obtain the value of haemoglobin in grms. per 100 mls.

$$\frac{\text{Reading of Unknown}}{\text{Reading of Standard}} \times 16$$

ENUMERATION OF RED CORPUSCLES

Apparatus

An improved Neubauer haemocytometer and red cell pipettes (British Standard 748) made by Hawksley, London, were used.

3% sodium citrate (99 mls.) and 1% formalin (1 ml.) was used as a diluting fluid. Dilutions of 1 in 200 were made.

Method of filling pipettes and counting chamber

After mixing the blood by rotating the bottle thoroughly between the palms of the hands, blood was drawn up exactly to the 0.5 mark and the pipette wiped clean. Diluting fluid was then drawn into the pipette up to the 101 mark. The filled pipette was shaken for 3 minutes at a slow speed on the Cresta pipette shaker (Hawksley).

The chamber was filled without allowing the pipette to stand after shaking. Filling was accomplished at a steady speed to get as even a distribution of cells within the chamber as possible.

In all cases duplicate red counts were made on a single blood sample. Two pipettes were used and both sides of the chamber filled. The blood was well mixed between the removal of each sample.

At least 2 minutes were allowed for the cells to settle and if the chamber was left longer than this it was placed in a covered petri dish containing cotton wool soaked in water. This prevented any evaporation.

Red /

Red cells were counted in 5 large (i.e. 80 small) squares. The squares at each corner and the one in the centre were selected for this purpose. Counting was performed under one-sixth objective in the usual manner, cells touching the left hand and upper lines of each square being included in the count. Those on the right hand and lower lines were excluded.

With a 1 in 200 dilution, four noughts were added to the total of erythrocytes counted in the 80 small squares. This gave the number of erythrocytes per cu.m.m.

ENUMERATION OF LEUCOCYTES

Apparatus

Again an improved Neubauer haemocytometer was used. 1 in 20 dilutions were made in leucocytes pipettes (British Standard 748) made by Hawksley, London.

Filling of the pipettes and haemocytometers was accomplished in the same manner as for the erythrocyte counts. Duplicate counts were done in all cases, two pipettes being used per sample.

The red cells lysed and the leucocyte nuclii stained with the gentian violet contained in the diluting fluid. Counts were done using a $\frac{2}{3}$ objective.

The composition of the diluting fluid used was as follows:- 2% solution of acetic acid (99 mls.) to which was added 1 ml. gentian violet.

Counting of Leucocytes

All the leucocytes in the 4 large 1 m.m. square areas at the corners of the chamber were counted. With the dilution of 1 in 20 the result was multiplied by 50 to give the number of leucocytes per cu.m.m.

DIFFERENTIAL LEUCOCYTE COUNTS

These were performed on the blood films after they had been stained and dried.

Many methods of counting have been described. The one used in this work was the "battlement system". In this method the cells are counted on or near the edge of the film. The edge of the smear was located and moved along two fields in a horizontal direction. Eight fields were then counted in a vertical direction followed by two fields horizontally and a further eight fields vertically until the same edge of the smear was again located. This procedure was repeated until all the cells had been counted.

Counts were performed on 100 cells at each of the four corners of the film, making a total of 400 cells counted per film. This ensured that the count was done evenly and covered all the areas of the film.

In a few cases only 200 cells were counted, this occurring when either the film was smaller than usual or the total white count was very low, so that it was impossible to locate 400 cells from the film.

The ~~e~~ method of classifying the various cells will be discussed later.

STAINING OF BLOOD FILMS

The routine stain used throughout this work was Leishman's (G.T. Gurr Ltd., London). The slide was placed on a staining rack. A known quantity of neat stain was applied to the unfixed smear and allowed to react for 2 minutes. This fixed the film.

Double /

Double this quantity of buffered distilled water (pH 6.8) was added to the stain and the two mixed using a Pasteur pipette. The stain was allowed to react for 15 minutes. Distilled water was applied to the film and left on for a few seconds and then the film was washed with a stream of distilled water until clean.

The slide was propped up on one end and the film allowed to dry in the air.

The stained film was a uniform rose-pink colour.

Other Staining Methods

These will be described later on under the appropriate section.

ENUMERATION OF PLATELETS

A considerable number of platelet diluting fluids were tried and found to be unsatisfactory for counting dog platelets. Finally, however, the fluid described by Baar (1948) was tried and found to be very satisfactory. This diluting fluid was used for all platelet estimations.

Baar's fluid is prepared as follows:-

Saponin B.D.H.	0.25 grms.
Sodium Citrate	3.50 grms.
40% Formaldehyde	1.0 mls.
Distilled Water to	100 mls.

No brilliant cresyl blue was included.

The number of platelets per cu.m.m. was worked out by multiplying the number of platelets counted in 80 small squares (1 in 20 dilution) by 1,000.

Apparatus

British Standard leucocyte pipettes (Hawksley) were used. Filling of pipettes and chambers was carried out in the same manner as for white cell counts.

The counting chambers were specially designed for plate-:let counting. They were half the thickness of the normal Neubauer haemocytometer. This allowed sufficient light, which was essential for accurate counting, to penetrate through the chamber. The ruling was the old Neubauer type.

Method for counting platelets

After filling, the chamber was allowed to stand in a covered petri dish for 30 minutes so that all the platelets settled.

Counts /

Counts were performed on 5 large (i.e. 80 small) squares under one-sixth objective. The four corner squares and one central square was selected. Duplicate counts were done as for red and white cell counts.

The platelets were seen as oval or round refractile bodies and were easily counted, provided continuous focusing up and down was performed during the count. Care was taken to count only platelets and to discount any irregularly shaped refractile bodies which sometimes appeared in the counting area.

DIRECT EOSINOPHIL COUNTS

These proved unsatisfactory unless the filling of the pipette was performed with blood taken directly from the vein and diluent added immediately.

Eosinophils were found to break up rapidly if the blood was left standing for an hour or more when non-siliconed bottles were used, Archer (1960). Even using siliconed bottles results were unreliable if left standing too long. For this reason comparatively few eosinophil counts were performed.

Method

Leucocyte pipettes were used and blood was drawn up to the 1 mark. The pipette was filled with diluting fluid to give a 1 in 10 dilution.

Counting was performed on a Fuchs Rosenthal chamber and all 16 squares were counted. Chamber depth was 0.2 m.m.

The number of eosinophils per cu.m.m. of blood was deduced from the following:-

$$\frac{\text{No. of eosinophils counted}}{\text{No. of squares counted}} \times 50$$

The diluting fluid, which proved satisfactory, was bromo-cresyl-purple, McNary (1958). This was made up as follows:- bromo-cresyl-purple 25 mgs. and distilled water 50 mls. As the diluting fluid lost its effectiveness after a few weeks fresh solutions were made up when required.

Counts were performed either on blood taken direct from a vein (usual method) or on blood from siliconed bottles.

The /

The latter method was only used when, for practical reasons, it was not possible to obtain blood directly from a vein.

PACKED CELL VOLUME ESTIMATIONS

A micro-haematocrit centrifuge (Hawksley) was employed for this estimation.

Method

The blood was thoroughly mixed in the bottle. Special capillary tubes, made for the purpose, were three quarters filled with blood. This was done by introducing the tube into the bottle and allowing blood to flow up the tube by capillary attraction.

The tube was wiped clean and one end sealed in a bunsen flame. Care was taken to ensure that no charring of the blood occurred and that the tube was properly sealed so the end was square.

After placing the capillary tube in the centrifuge it was spun for 5 minutes at maximum revolutions which develop a force of 12,000 x g. The centrifuge switched itself off when this period of time had elapsed. The packed cell volume was read off on a special micro-haematocrit reader (Hawksley).

SEDIMENTATION RATE ESTIMATION

Wintrobe tubes of 3.0 m.m. diameter were used. These were scrupulously clean. By using a Pasteur pipette, they were filled to the 100 m.m. mark with blood (thoroughly mixed). This was done as soon after collection of the blood as possible. No air bubbles were allowed to enter the column.

Readings were taken after the tubes had stood in a vertical position for one hour. A special stand fitted with a spirit level (R.B. Turner Ltd., London) was used to hold the tubes.

RETICULOCYTE COUNTS

These were performed on blood from the non-siliconed bottles. The stain used was prepared as follows:-

3%	Sodium Citrate	20 mls.
0.85%	Sodium Chloride	80 mls.
Brilliant Cresyl Purple		0.4 grms.

The stain was filtered immediately prior to being used.

Method

Two or three mls. of stain were placed in a centrifuge tube. Four or five drops of blood were added to this, and the tube was incubated in a vertical position at 37°C for a period of 30 minutes. This facilitated penetration of the stain into the red cells.

After removal of the tubes from the oven, the supernatant stain was poured off. The concentrated red cells at the bottom of the tube were sucked up with a Pasteur pipette and a drop placed on a clean grease free slide. A thin film was made from this drop with the use of a spreader.

After the film was dried it was counted under a $\frac{1}{12}$th. oil immersion objective. One thousand red cells were counted and the number of reticulocytes in these thousand cells was calculated.

A special diaphragm eye-piece was used, by means of which the microscope's field could be reduced as desired to facilitate counting.

Reticulocytes were red cells which had a dark blue reticular network within them. Mature red cells stained uniformly /

uniformly green. Cells which stained a uniform blue colour were not considered to be true reticulocytes.

METHODS USED FOR DETERMINING CORPUSCULAR VALUES

The following formulae were used for the calculation of the various corpuscular values:-

Mean Corpuscular Volume:

$$\frac{\text{Packed Cell Volume per cent} \times 10}{\text{Red cells in millions per cu.m.m.}} = c\mu$$

Mean Corpuscular Haemoglobin:

$$\frac{\text{Haemoglobin in grams per litre of blood}}{\text{Red cells in millions per cu.m.m.}} = \mu\mu \text{ grms.}$$

Mean Corpuscular Haemoglobin Concentration:

$$\frac{\text{Haemoglobin in grams} \times 100}{\text{Packed cell volume per cent.}} = \%$$

In the later stages these values were calculated by means of a Camlab haematological slide rule designed by J.W. Nicholas.

SPECIFIC GRAVITY DETERMINATION

Serial solutions of copper sulphate were used for this. Blood was allowed to drop from a height of 2 cms. above the solution into the bottle of copper sulphate. A Pasteur pipette was used for this purpose. The drop becomes coated with copper proteinate and remains discrete for up to 20 seconds.

If the drop of blood sank in the solution, the specific gravity of the blood was higher than that of the copper sulphate. If it rose, the specific gravity was lower, and if it remained stationary for a period of about 10 seconds the specific gravity of the blood and the copper sulphate solution were identical.

By using a series of bottles with specific gravities ranging between 1.036 and 1.075 the specific gravity of any blood sample could be ascertained easily, quickly and accurately.

Each bottle of copper sulphate solution remained accurate provided no more than 100 drops of blood had been introduced into it. After this inaccuracies occurred. For this reason fresh solutions were made up from time to time.

The solutions of copper sulphate were made up following the method of R.A. Phillips.

A solution of copper sulphate of specific gravity 1.100 was prepared by dissolving 159 grms. copper sulphate in 1,000 mls. of water at 25°C. The accuracy was checked by weighing a bottle empty; containing 25 mls. water; and lastly containing 25 mls. copper sulphate. Weight of the solution divided by the weight of water should be 1.100 if the solution was accurately prepared.

Solutions /

Solutions with specific gravities between 1.036 and 1.075 were prepared. For specific gravity 1.036 dilute 35 mls. of 1.100 copper sulphate solution with water to 100 mls. For specific gravity 1.037 dilute 36 mls. with water up to 100 mls. This procedure was carried on up to 1.075 in which 74 mls. solution were diluted to 100 mls. with water.

RED CELL FRAGILITY

A modification of the method described by Parpart, Lorenz, Parpart, Gregg & Chase (1947) was used.

METHOD

A stock solution of 1% sodium chloride was prepared and from this saline solutions ranging from 0.275% to 0.750% were made up. The variation between each solution was 0.025%. A solution of 0.85% saline was prepared and used as the blank, and an 0.04% ammonia solution used as the 100% haemolysis standard.

A series of tubes containing the various dilutions of saline were prepared, eight millilitres of solution being placed in each tube. The blood was placed in a petri dish and agitated gently until a bright red in colour and then 50 cu.m.m. of blood was introduced into each tube and mixed by sucking the solution in and out of the pipette several times. Blood was introduced into the tubes containing 0.85% saline and 0.04% ammonia in an identical manner, after the saline tubes had been filled.

The tubes were left standing at room temperature for 45 minutes to allow complete haemolysis to take place and then centrifuged for 15 minutes at 1,500 r.p.m.

An EEL colorimeter, with a 404 filter, was used to ascertain the degree of haemolysis in each tube. Matched tubes were used in the machine which was first zeroed using 0.85% saline.

Each solution was poured into a matched tube, the reading taken, and the solution discarded. The next solution was poured into /

into the same matched tube, the reading taken and the solution discarded. This procedure was repeated until all the readings had been completed. The blank followed by the ammonia was the last to be read.

The readings obtained were compared with those from the blank (no haemolysis) and the 100% haemolysis standard to find the percentage haemolysis in each tube.

CLOTTING TIME ESTIMATIONS

Clotting time was ascertained using a modification of the Lee and White technique, described to me by J. Watt (1962).

Method

A clean, dry, non-siliconed 1 c.m. diameter glass tube was used. Blood was allowed to fall direct from the needle (not siliconed) into the tube, and a stop watch started as soon as the first drop entered the tube. Two millilitres of blood were collected.

A cork (not siliconed) was placed in the top of the tube and the tube was then either held tightly in one hand or placed in a trouser pocket. This allowed the temperature to remain approximately at a standard temperature. (All estimations were carried out indoors where the temperature was stable). This procedure was adopted as it was quite impossible to take a water bath to some of the places where dogs were bled.

Every 30 seconds the tube was inverted gently.

Clotting /

Clotting was considered to be complete when the clot stuck to the bottom of the tube after inversion. At this point the watch was stopped and the time taken for clotting to occur was recorded.

BLOOD PICTURE OF THE NORMAL DOG

INTRODUCTION

During the more recent years great advances have been made in the clinical diagnosis and treatment of various disease processes both in the large and small animal fields. Amongst the small animals, the dog holds a prominent position, being one of the most popular pets in this country to-day.

In order to aid in accurate diagnosis, laboratory techniques are becoming widely used, and one of these techniques is the examination of the patient's blood for abnormalities. It is, of course, obvious that for these tests to be of any value, the normal variations occurring in dogs' blood must be known accurately. As the dog is also widely used as an experimental animal the need for accurate information on normal values becomes doubly important.

On reading the considerable literature available on the subject of the normal blood picture in dogs, it became very obvious indeed that there were large gaps in this literature. The figures quoted in various text books and articles showed a wide range of normality and in many cases did not help in assessing the values of the blood picture in a particular case.

Although some emphasis has been laid recently on the blood picture of young and old dogs and on some aspects of the variations due to sex, little or no attention has been paid to other physiological conditions which might affect the blood picture. /

picture. Variations due to breed, those due to different systems of nutrition and environment, to pregnancy, oestrus cycles, feeding, exercise, travelling, lack of water or slight degrees of parasitism have been dealt with either by one or two authors rather inadequately, or not at all.

The object of this work is to fill these gaps in our knowledge, to consolidate the information already available, and to consider as many aspects of the blood picture as possible in order to arrive at some standard of normality. The work will be primarily directed towards variations which are clinically significant in the hope that the results obtained will help clinicians to discern normal variations in the blood picture of dogs from pathological variations. No biochemical estimations will be included in this work.

The blood of healthy dogs has been examined and a var:iety of estimations carried out. These estimations include total red cell counts, total white cell and differential white cell counts, as well as haemoglobin estimations, packed cell volumes, reticulocyte counts, sedimentation rates, specific gravities and platelet counts. Corpuscular values have also been calculated and fragility and clotting times estimated. Cell diameters were not calculated as these were thought to be of little importance from a clinical standpoint. A description of all the types of cells encountered in normal blood will be given and special mention is to be made of the characteristics of canine eosinophils.

REVIEW OF THE LITERATURE

The literature available is extensive, but in every case incomplete, and it is difficult to establish accurately true normal blood values from them.

The literature can be divided into three main sections: prior to 1910; 1910-1940; and after 1940 - the first section being of historical interest only.

PRIOR TO 1910

The first account I have located which deals with the blood of normal dogs is that by Vierodt (1854). He gives red cell counts for two normal bitches, one a Dachshund and the other a Spitzhound. The samples were removed prior to experimentation on the dogs and the article was not intended as an investigation of the normal values. The diluting fluid used was a mixture of 2.25 grms. of sugar and 0.16-0.17 grms. of table salt made up in 100 c.c. of water. No counting chambers were used. His results were as follows:-

Dachshund - 4,612,000 per cu.m.m. (mean of two counts)
Spitzhound - 4,231,000 per cu.m.m.

These results appear to be surprisingly accurate considering the equipment he had at his disposal.

Several articles were written between 1854 and 1890, mostly by continental authors. The Germans appearing to be particularly active in this field.

In /

Otto (1885) did considerable work on red cell and haemoglobin values in men, rabbits and dogs. He used seventeen dogs (twelve male and five female). His average value for the erythrocyte counts was 6,000,000 per cu.m.m. and the range 8,977,000-4,039,000 per cu.m.m. He considered males to have slightly higher erythrocyte counts than females. The haemoglobin average was 14 grms. per 100 mls. and the range 12.06-15.98 grms. per 100 mls. Hüfner's spectrophotometer was used in the estimation of haemoglobin values.

In 1894 Sherrington's paper "Note on some changes in the blood of the general circulation consequent upon certain inflammation of acute and local character", was published. In this paper he describes the results obtained from normal dogs prior to experimentation.

Thoma-Zeiss chambers and Hawksley pipettes were used. Haemoglobin estimations were done using Gower's instrument and specific gravity by the method of Roy.

The red counts range from 2,502,000-8,126,000 per cu.m.m.; white counts from 6,260-12,610 per cu.m.m. and haemoglobin from 47-72%. Specific gravities ranged between 1.0515 and 1.066. In a young dog the specific gravity was 1.054 before feeding and 1.054 three hours after feeding.

Sherrington also describes the morphology of the cells. These results were obtained from six dogs; age, breed and method of management was not discussed.

In /

In 1894 Zenoni also gave the blood values for normal dogs prior to experimentation. The dogs used were young but again no exact age or breed is quoted.

Thoma-Zeiss chambers were used. The diluting fluid for the white cells was a 1 to 300 solution of acetic acid and water, while that used for the red cells was a 3% sodium chloride solution.

Haemoglobin was estimated using a Bizzozero chromocytometer. The results obtained were:-

Red blood cells	6,150,000 per cu.m.m.
White blood cells	7,000 per cu.m.m.
Haemoglobin	97%

The counts of white cells stained haematoxylin-eosin were as follows:-

Polynuclear Leucocytes	62%	Absolute numbers	4,350 per cu.m.m.
Mononuclear Leucocytes	28%	Absolute numbers	1,900 per cu.m.m.
Eosinophil Leucocytes	10%	Absolute numbers	760 per cu.m.m.

It can be noted that even at this early date acetic acid was being used as a white cell diluting fluid and that absolute figures for differential white cell counts were being used as well as percentage figures. Counting chambers were also in use.

In 1900 Tallqvist and Willebrand described the morphology of canine white cells and gave the results from fifteen dogs of unknown age or sex. White cells range from 8,100-15,800 per cu.m.m. (average 12,400 per cu.m.m.). The mean differential counts of these dogs can be summarised as follows:-

Name of Cell	Percentage
Neutrophils	75.0
Eosinophils	5.2
Monocytes	13.4
Lymphocytes	6.1

Also in 1900 Dawson bled a series of dogs and then injected various saline solutions. He recorded the values for fifteen dogs prior to the experiments. Blood was obtained from a slit made in the margin of the ear.

A Thoma-Zeiss chamber was used for cellular counts; Von Fleischl's haemoglobinometer for haemoglobin determinations, and the smears were stained Ehrlich's triacid and haemotoxylin-eosin.

The red cell counts ranged from 6,250,000-8,500,000 per cu.m.m. with an average of 7,215,000. Red cell diameters were 6-8 µ.

Haemoglobin in twelve dogs was 59-98%, the average being 75%. White cell counts varied from 11,000-28,000 per cu.m.m. with an average of 19,000 per cu.m.m. The range of variation and average differential results for white cells in ten dogs can be summarised as follows:-

Type of Cell	Range of Variation	Average Percentage
Neutrophils	62.4% - 68.0%	64.25
Lymphocytes	11.2% - 31.6%	22.17
Eosinophils	2.6% - 21.6%	8.85
Other Forms	1.2% - 9.4%	4.42

Mezincescu (1902) gives the results of red, white and differential white counts from four dogs prior to removal of the thyroid gland.

A /

A Thoma-Zeiss chamber was used for cellular counts. He gives approximate ages for the dogs but no indication of breed. The differential results do not add up to 100% in all cases, and in one case total 104.5%. Little significance can be placed on this last result.

His results are given below:-

Dog and Age	Erythrocyte count per cu.m.m.	Leucocyte count per cu.m.m.	Polynuclears %	Large Mononuclears %	Lymphocytes %
Dog 3-4 months	4,100,000	9,100	70	18	10.5
Aged Bitch	6,012,500	8,750	72	17.5	9.4
Dog 4 months	4,890,000	10,825	68	22.1	9.2
Adult Dog	7,700,000	12,500	69	25.5	10.0

This is the first work in which age has been noted although he makes no attempt to correlate his results with the dog's age.

Busch & Van Bergen (1902) used twenty normal dogs and bled them at least twelve hours after feeding. No attempt was made to class them according to age, sex or breed.

Their results can be summarised in the following table:

Number of Dogs	Erythrocytes per cu.m.m.	Leucocytes per cu.m.m.
20	4,225,000 - 8,030,000 Average 6,206,000	7,200 - 14,375 Average 9,526

Polymorphonuclear %	Lymphocytes %	Large Mononuclear %	Eosinophils %	Basophils %
65.7	21	6.8	5.3	Rare

They also describe the morphological characteristics of the cells. They mention that lymphocytes may have double nuclei that the nucleus may be pyknotic, or may have a "mural" type of nucleus (central clear area in the nucleus). Neutrophilic granules may also appear in a lymphocytes cytoplasm. The commonest type of neutrophil is one with a nucleus which is not completely segmented. The granules of the eosinophils varied in size. Such attention to morphological detail is often absent in more recent publications.

Nicolas & Dumoulin (1904) gave eleven results from two normal dogs prior to splenectomy. Only total white and differential white cell results were given. The average results were:-

Dog	Leucocytes per cu.m.m.	% Polynuclears	% Lymphocytes	% Mononuclears	% Eosinophils
No. 1	9,648	68	10.8	17	3.5
No. 2	9,750	77	10.0	9	2.0

Discrepancies in the percentage totals may be due to a failure to include miscellaneous cells.

In 1905 work on normal dogs by Burnett and Traum was published. Eleven normal dogs were used. Blood was obtained from the ear. Toisson's fluid was used to dilute both red and white cells. A Thoma haemocytometer with Zappert-Ewing ruling was employed for counting and haemoglobin was estimated using Gower's haemoglobinometer. The results for each dog are listed separately and an approximate age is given with each. No mention is made of breed or system of management. Only a summary of their work is possible here.

Red cell counts per cu.m.m.	5,656,000 (young dog)	6,560,000 ($1\frac{1}{2}$ years)
White cell counts per cu.m.m.	10,520	5,864
Haemoglobin %	108	77

Nucleated red cells were seen in all but four dogs.

The average differential results are given below.

Polynuclear per cu.m.m.		Lymphocytes per cu.m.m.		Monocytes per cu.m.m.		Eosinophils per cu.m.m.	
7,800-3,874		2,762-743		1,030-370		828-110	
percentages		percentages		percentages		percentages	
highest	lowest	highest	lowest	highest	lowest	highest	lowest
76	57.9	29.1	11	10.4	3.1	10.5	1.4

They /

They also include the results from seven puppies (six male and one female) aged between one and twenty days, and give comprehensive information for each puppy. The average results were:-

Erythrocytes per cu.m.m.	Haemoglobin %	Leucocytes per cu.m.m.
3,771,000	81	8,622

Average results for differential leucocyte counts:-

Polynuclear %	Lymphocytes %	Monocytes %	Eosinophils %
58.5	23.8	7.9	9.8

Absolute figures were also given for each dog.

They noted that normoblasts were present in large numbers in these puppies. They were highest in the one to six day old group, and ranged from 1,589 per cu.m.m. to 54 per cu.m.m.

Pregnant bitches were also bled (see later under pregnancy section).

This work was the most comprehensive up to this time, and correlated the blood picture with age, although relatively few dogs were examined. The picture seen in puppies was given for the first time and could be compared with the results for adult dogs.

Sabrazès /

Sabrazès and Muratet (1906) gave their results for two normal dogs of unknown age and sex. This article dealt with the changes seen in distemper and added nothing to the information already available on the blood of normal dogs.

Wetzl (1910) gave the results of the red counts and haemoglobin values for five dogs (three female and two male). The dogs were aged one or two years. Blood was obtained by cutting an ear vein. Haemoglobin was estimated using Tallqvist's method and also on a Fleischl's haemometer. Breed and method of management were not given.

Red counts ranged from 4,816,000-8,124,000 per cu.m.m. (Average 6,240,000 per cu.m.m.). Haemoglobin (Tallqvist) ranged from 100-60% and using the haemometer from 55-104%.

Goodall (1910) gave results for red cell counts, haemoglobin, total white and differential white cell counts, but the results are of doubtful value as his average figures were higher than the extremes of his range. He does state, however, that the blood picture varied with age, feeding and environment.

PERIOD 1910-1940

The first account of the blood picture of normal dogs reviewed in this section is by Wells and Sutton (1915). Three puppies (three to seven days old); three dogs, and eight bitches (bled two to one hundred and fifty-one days after parturition) were used. All were bled more than once by cutting an ear vein. The dogs were of unknown age (apart from the puppies) and all housed in laboratory kennels. The breeds are unknown.

Average /

Average results were as follows:-

Type of Cell	Puppies	Adults
Erythrocytes per cu.m.m.	4,268,000	6,709,000
Leucocytes per cu.m.m.	16,290	11,000

The average red cell counts for adults must have been lowered as bitches were used just after parturition when they cannot be considered normal. (see pregnancy section).

They also noted changes after exercise and pregnancy (see later under appropriate section).

Musser and Krumbhaar (1916) did fragility tests on twenty-two normal dogs using solutions of saline. They found haemolysis started at 0.462% and is complete at 0.330%.

Burnett (1917) produced a very comprehensive book in which he gave the results of numerous authors. The results quoted show how wide a normal range there was, and how little information was available to account for them at this time. His results are summarised as follows:-

	Haemoglobin %	Erythrocytes per cu.m.m.	Leucocytes per cu.m.m.
Maximum	110.0	8,000,000	31,000
Average	88.6	6,093,000	12,168
Minimum	55.0	4,000,000	6,000
Average - Male	-	6,100,000	-
Average ⊖ Female	-	5,700,000	-

Differential white cell results:-

	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Maximum	78	29	10	10.5	0.5
Average	68	20	6	6.0	rare
Minimum	60	11	3	1.0	rare

In 1927 Stebbins and Leake did specific gravity estimations on six dogs using the falling drop method of Barbour and Hamilton. They showed that diurnal variations occurred. Their range of results was 1.0453-1.0644.

Kohanawa (1928) produced a long article which dealt with dogs and also other species of domestic animals. Twelve dogs were used in his work (seven male and five female), and ages varied from one to three years. No data is given on breed, feeding, or method of management.

Blood was obtained from a cut in the ear flap. Red cells were diluted with Toisson's fluid and Turk's fluid was used for dilution of the white cells.

The results are summarised as follows:-

Mean Erythrocyte count per cu.m.m.	Range of Erythrocyte count per cu.m.m.	Mean Leucocyte count per cu.m.m.	Range of Leucocyte count per cu.m.m.
6,206,000	5,240,000 to 7,16o,000	17,050	12,800 to 19,800

Mean differential Leucocyte counts:-

Neutrophils	Lymphocytes Small	Lymphocytes Large	Monocytes	Eosinophils	Basophils
77.4%	1o.7%	2.5%	6.1%	3.3%	None seen

He gave details of all the cell types seen, and their diameters. 5.9% of small lymphocytes and 3% of large lymphocytes had azurophilic granules. He counted an average of 39 platelets per 1,000 red cells on the blood films. This works out at 234,000 platelets per cu.m.m. This is the first article I have seen which mentions platelet counts.

In the year 1930 at least three publications were produced on normal dog's blood. Mayerson (1930) did one hundred and seventy-eight determinations on sixty normal dogs. Both males and females were used. Ages and breeds are not specified. All were wormed, kennelled, and fed a standard diet. He states that his work was done to stimulate others to complete a study on normal dogs so that standard values could be arrived at. All cellular counts were done in duplicate. Platelets were counted using Rees-Ecker diluting fluid (fifty-six estimations).

Haemoglobin was estimated by the use of a Newcomer haemoglobinometer. Reticulocytes were stained with cresyl blue and counterstained with Wright's stain, three hundred cells being counted (thirty-four estimations).

Haematocrit /

Haematocrit was determined by centrifuging Van Allen haematocrit tubes for half an hour at 3,200 revolutions per minute.

Fragility was by the method of Silvette (thirty-four estimations).

For specific gravity he used the method of Barbour and Hamilton (thirty-eight estimations).

Results are given in the table:-

Observations	Average	Range of Variation
R.B.C. per cu.m.m.	6.16 million	4.86 - 8.56 million
Reticulocytes	1.85% (113,960 cells observed)	
Haemoglobin per 100 mls.	13.01 grms.	9.37 - 15.6 grms.
Corpuscular Haemoglobin	20.0 grms. x 10^{-12}	25.8 - 15 grms. x 10^{-12}
Corpuscular Volume	59.3 cu.µ.	81 - 41.4 cu.µ.
Haematocrit %	38.6	49 - 26
Specific Gravity	1.0503	1.0420 - 1.0562
Fragility		0.5% - 0.8%
Platelets per cu.m.m.	621,000	1,275,000 - 370,000
White Cell per cu.m.m.	11,165	19,200 - 5,650

The /

The percentage differential leucocyte counts can be summed up as follows:-

Range and Average	Polymorphs	Lymphocytes	Mononuclear	Eosinophils	Basophils
Range of Variation	56-89	9-34	1-10	0-7	0-1
Average	74	20	4	2	0

This work is the first reviewed to give normal results for packed cell volume, reticulocytes, corpuscular haemoglobin, and corpuscular volume. From this viewpoint it was a good addition to the information already available in 1930.

Powers, Bowie and Howard (1930) studied twenty-five healthy young male dogs with an average weight of 14.53 kgms. No age is given. Blood was obtained from the saphenous vein and the anticoagulent was sodium oxalate.

Haemoglobin was estimated by the Sahli method and haematocrit by centrifuging the blood for half an hour at 3,000 revolutions per minute. The results were given according to the weight of the animal.

The average results for all the dogs irrespective of weight are as follows:-

Erythrocytes	7,002,000 per cu.m.m.
Haemoglobin	92.8%
Haematocrit	45.21%

Alexandrov (1930) used twelve normal dogs for his study. The sexes are given and a vague description of the breed, e.g. "young /

"young dog, woolly coat". Figures for age are given only very approximately and in some instances are absent.

His mean results are given in the tables.

Haemoglobin (Sahli)	Erythrocytes per cu.m.m.	Leucocytes per cu.m.m.
100%	6,335,000	11,260

Meta. %	Seg. %	Eosin. %	Baso. %	Lymph. %	Humprecht and Broken Cells %	Mono. %	Retic. Endo.%	Türk %
0.17	67.23	3.48	0.06	14.3	1.3	12.3	0.02	1

He is the first person to record the presence of Humprecht cells, Türk cells, and reticulo-endothelial cells in the peripheral blood of normal dogs.

Scarborough (1931) published work on normal dogs. He lists a great number of authors, over 600 in all, and gives the average results obtained by each. It is not possible to list his findings here, but some of the more interesting features will be included. He lists several authors who have noted reticulocytes in the blood, two who have noted an increase in the red cells and haemoglobin with increasing age, and four who consider males have a higher red cell count than females. All these features have already been noted in this review.

He also notes that environment, feeding and emotional stimuli /

stimuli causes variation in the red cell picture. He gives no reference to substantiate these statements.

He also states that the white cell count increases after feeding, is higher in puppies than in adults, and that neutrophils fall in pregnancy. He gives references to back up these statements. No references are given, however, when he says that the white cell count does not vary with breed.

The physiological variations will be dealt with in full later in this work.

Scarborough added little to the existing literature.

Leichsenring and Hönig (1931) used seven normal dogs prior to experimental bleeding. All were kennelled, and no age or sex was given. Red cells were diluted with Hayem's fluid and duplicate counts were performed.

Results:

Erythrocytes per cu.m.m. 7,200,000 (6,430,000 - 7,890,000)

Packed cell volume % 54.9 (46.1 - 61.8%)

In 1932 Leichsenring, Biester, Hönig, Furnas, Foss and Routt used thirty-two normal dogs (sixteen male and sixteen female). The methods used were as above. No age or breed given. The results were classed according to sex and the weights were recorded.

They found no sex variations.

Sex	Erythrocytes per cu.m.m.	Haemoglobin grms. per 100 mls.	Leucocytes per per cu.m.m.	Average Weight
Males	7,170,000	14.11	8,490	14.42 kgms.
Females	7,170,000	14.11	9,090	17.24 kgms.

Wintrobe, Schumacker and Schmidt (1936) used fifty-four adult mongrel dogs, weighing 5-17 kgms. for a study of normal blood values. All were kennelled and fed a standard diet. Blood was collected from the jugular vein into ammonium and potassium oxalate. Duplicate counts were performed.

Haemoglobin was estimated using a Newcomer haemoglobinometer.

The following is a synopsis of their results:-

Erythrocytes per cu.m.m.	Haemoglobin grms. per 100 mls.	Packed Cell Volume %	M.C.V. cμ.	M.C.H. μμg.	M.C.H.C. %
7,020,000	14.6	47.3	67.6	21.2	31.4

Variations due to sex were as follows:-

Sex	Erythrocytes per cu.m.m.	Haemoglobin grms. per 100 mls.
22 Males	7,160,000	15.1
10 Females	6,780,000	13.9

These variations in red counts and haemoglobin values between the two sexes do not appear significant.

Bruner and Wakerlin (1937) also studied kennelled, adult mongrel dogs in their work. Thirty-four dogs were used (seventeen males /

males and seventeen females). Blood was taken from the saphenous vein and heparin used as an anticoagulant. Wintrobe tubes were used for packed cell volume estimations and Newcomer's method for haemoglobin. Reticulocyte counts were done on 1,000 cells and red cell counts were done in duplicate.

Statistical calculations were performed and these results are given here:-

	Mean	Standard Deviation	Coefficient of Variation
Erythrocytes millions per cu.m.m.	6.45 ± .03	0.76 ± .02	11.73
Haemoglobin grms/100 mls.	13.56 ± .07	1.60 ± .05	11.84
Haematocrit %	44.28 ± .20	4.78 ± .14	10.79
M.C.V. cμ.	68.9 ± .19	4.53 ± .14	6.58
M.C.H. μμ.grms.	21.1 ± .07	1.57 ± .05	7.44
M.C.H.C. %	30.7 ± .06	1.40 ± .04	4.56
Reticulocytes %	0.44 ± .014	0.42 ± .010	95.7
Leucocytes thousands per cu.m.m.	14.18 ± .22	5.21 ± .16	36.75

Differential leucocyte counts were not done. No significant sex difference was found.

Tocantins (1938) in his long article on mammalian platelets reported that the range in fifty-three dogs was 188-960,000 per cu.m.m. with an average of 461,000 per cu.m.m.

The last article in the period was by Landsberg (1939). He used mongrel dogs (litter mates) all kennelled and fed identically. Ninety-five dogs were used (thirty-eight male and fifty-seven female). The mean age of the dogs was one hundred and two days and the mean weight 4.5 kilos. The dogs were bled by cardiac puncture, and multiple bleeding was carried out.

Haemoglobin was estimated using Newcomer's haemoglobinometer; packed cell volume using Wintrobe tubes, and differential smears were prepared direct from the needle.

A summary of the results follows:-

Average results and Standard Deviations

Sex	Erythrocytes millions per cu.m.m.	Haemoglobin grms. per 100 mls.	P.C.V. %	M.C.V. cu.μ.	M.C.H. μμ.grm.	M.C.H.C. %
Male	4.810 ± 0.98	8.68 ± 0.22	36.3 ± 0.59	76.0 ± 0.894	18.84 ±0.44	24.63 ± 0.58
Female	5.119 ± 0.102	9.14 ± 0.24	38.8 ± 0.63	76.5 ± 0.74	18.51 ± 0.31	24.2 ± 0.40

Average /

Average total white counts and results of differentials are also given:

W.B.C. per cu.m.m.	Myelo. %	Meta. %	Neut. %	Lymph. %	Mono. %	Eosin. %	Baso. %
11,895	1.30	4.37	63.61	28.13	0.70	1.81	0.03

The average reticulocyte count was 1.36% and the average platelet count 154,879 per cu.m.m. His results were given as frequency distribution charts. This is the first article in which these have appeared.

Although not in this group the work of Landsberg (1942) is included as a comparison with the work just described. For this he used sixty-six mature mongrel dogs (forty-one male and twenty-five female) and again all were kennelled and fed standard diets. Bleeding was from the jugular vein and Heller and Paul's anti-coagulant was used. The estimations were done in the same way as the work of 1939.

The mean results are set out in the following table, together with the standard deviations:-

	Male	Female	Combined Results
Erythrocytes millions per cu.m.m.	6.28 ± 0.119	6.15 ± 0.158	6.27 ± 0.97
Haemoglobin grms/100 mls.	13.8 ± 0.351	14.1 ± 0.313	13.9 ± 0.233
P.C.V. %	44.4 ± 0.867	43.6 ± 0.692	44.2 ± o.661
M.C.V. cu.µ.	69.3 ± 0.840	71.3 ± 0.108	70.55 ± 0.692
M.C.H. µµ. grms.	22.6 ± 0.487	21.2 ± 0.502	21.8 ± 0.347
M.C.H.C. %	32.4 ± 0.522	32.2 ± 0.524	32.3 ± 0.384

These results show that adult dogs have higher red counts, haemoglobins and packed cell volumes than young dogs. Their erythrocytes also tend to be smaller. Males have higher red counts than females but the results given do not show the difference to be significant.

PERIOD 1940 - PRESENT TIME

Although much work had already been done on the blood of normal dogs the picture as a whole was far from complete and many articles appeared after 1940 in an attempt to rectify this situation.

Morris, Stelton, Allison and Green (1940) used sixty-six normal dogs. Most of these were puppies coming in to the Veterinary Hospital for distemper inoculations. Blood was taken from the radial vein. Improved Neubauer chambers were used for counting. The counts were performed by eight different people.

The /

The sex, age and breed of the dogs is given. Only red cell counts, haemoglobin, white cell counts and differential white cell counts were performed (100 cells only) as shown in the following tables which give their average results.

Dogs	Erythrocytes millions per cu.m.m.	Haemoglobin grms/100 mls.	M.C.H. $\times 10^{-12}$ grms.	Leucocytes per cu.m.m.
2-8 months	5.3	12.6	24	12,165
Adult	6.2	15.1	24.3	11,467
Males	6.46	-	-	-
Females	6.57	-	-	-

Differential leucocyte counts were given as follows:-

Dogs	Seg. %	Stab. %	Lymph. %	Eosin. %	Mono. %
2-8 months (35)	55.85	6.7	33.3	3.97	0.15
Adult (31)	65.27	6.56	21.72	5.43	0.67

They considered that red cells and haemoglobin increased with age, but that age, sex, and breed did not affect the white counts.

Adler (1940) used one hundred dogs aged between three to ten months. These were pets coming in to hospital for distemper inoculations. Sodium citrate (3.8%) was used as an anticoagulant. Haemoglobin was estimated by the acid haematin method, and improved Neubauer chambers were used for counting.

The /

The average results were:-

R.B.C. per cu.m.m.	Haemoglobin grms/100 mls.	W.B.C. per cu.m.m.	Stabs. %	Seg. %	Lymph. %	Eosin. %	Mono. %
5,500,000	11.2	12,240	5.37	50.55	37.77	5.79	0.50

It was considered that age, sex and breed had no effect on the red count, and that young dogs have higher white counts than adults. Young dogs have fewer neutrophils and more lymphocytes than adults.

Simms (1940) studied the sedimentation rates in eighteen normal mongrel dogs aged from eight weeks to adult. The rate was 1-4 m.m. per hour. In pregnant bitches the rate increased to 1-52 m.m. per hour (average 15 m.m.). The anticoagulant used was sodium citrate.

Mundhenk (1941) wrote three articles dealing with variations in the blood of normal dogs. Over two hundred dogs (mostly mongrels) were examined. One third were from the pound; one third were suffering from minor ailments, and the rest were pets and strays. Ages varied from one to nine years and as no de-worming was done, many dogs had intestinal parasites.

Blood was obtained from a cut in the ear flap. A large number of estimations were carried out and the results are given below.

Haemoglobin /

Haemoglobin averaged 84.2% using the Tallqvist haemoglobinometer, and the range was 40-100% . Fifty-six males and forty-four females were used but no sex variation was noted.

Red cells averaged 6,412,000 per cu.m.m. (both sexes) and the range was 2,440,000-11,016,000 per cu.m.m., an enormous range. The lowest count was from a dog heavily infested with hookworms.

White cell counts ranged from 2,700-21,100 per cu.m.m. with an average of 8,867 per cu.m.m. One hundred dogs were used in this part of the work.

Differential white cell counts were:-

	Neuts. %	Lymph. %	Mono. %	Eosin. %	Baso. %
Maximum	86	44	18	24	2
Average	64.6	19.28	8.86	7.0	0.2
Minimum	39	4	2.0	0	0

(Arneth indices were calculated and Schilling counts were also done).

The results from three dogs bled every two hours over a period of twenty-four hours was given. This showed that fluctuations occurred in the blood picture throughout the whole day.

In the conclusion he states that "lack of food or water, salting and consequent over-drinking, over-feeding, oestrum, parturition, suckling of puppies, parasitisms, gastro-intestinal irritation, the action of certain drugs or foods, weather conditions, exercise or lack of it, excitement, altitude, temperature and various factors probably affect the haemograms of apparently normal animals".

He /

He gives no positive data or figures to support any of this statement.

This work covers a lot of ground, involves results from a large number of dogs, gives no indication of the exact age or breed of the dogs used, and is consequently of far less value than it could have been. It is very doubtful if the enormous variations in cell counts given in this article would occur in really healthy normal dogs unless they were undergoing some form of physiological stress such as pregnancy, or were housed in large kennels.

Mulligan (1941A) studied sixty adult mongrel dogs living at an altitude of one mile. Thirty-nine were males and twenty-one females, and weights varied from 5-19 kilograms. Red cell counts ranged from 5.22 millions to 8.46 millions per cu.m.m. (average 6.79 millions per cu.m.m.). Haemoglobin values were 11-18.8 grms. per 100 mls. and averaged 14.5 grms per 100 mls. while the mean corpuscular haemoglobin was 21.5 $\pm$ 2.45 μμ grms.

White cell counts were 13,150 $\pm$ 5,250 per cu.m.m. The range of variation and average results of the differential counts are given in the table.

Stabs. %	Seg. %	Lymph. %	Eosin. %	Mono. %
0.5-10 Average 4.3	54.5-91.5 Average 73.9	4.5-37 Average 17.1	0 -13 Average 3	0 - 7 Average 1.7

He found no relationship between haemoglobin or red counts and weight or sex.

Mulligan (1941B) gave the results for twenty-seven dogs of unknown age, living at a normal altitude.

R.B.C. per cu.m.m.	Haemoglobin grms. per 100 mls.	W.B.C. per cu.m.m.	Stabs. %	Seg. %	Lymph. %	Eosin. %	Mono. %
6.76	14.4	10,700	4.2	70.6	19.3	4.1	1.8

He also gives results for five puppies aged two to seven months. The only positive finding was that red cells were low in young puppies - 3.92 million per cu.m.m. - as compared with 5.93 million per cu.m.m. in the seven month puppy.

Crandall, Finne and Smith (1941) did forty-nine observations on twenty-five normal dogs of unknown age, sex and breed. They gave no new information.

Handler and Featherstone (1943) give the normal values for twenty-eight dogs of unknown age or breed, prior to feeding them on different diets.

Results are given for two groups of dogs, each group consisting of fourteen dogs.

Average weight of dogs	R.B.C. millions per cu.m.m.	Hb. grms per 100 mls.	Retic. %	P.C.V. %	M.C.V. cu.μ.	M.C.H. μμ.gms.	M.C.H.C. %
10.7 kgs.	6.9 ± 0.46	15.2 ± 1.6	<1	45.5 ± 6.1	66	22	33
10.2 kgs.	7.24 ± 0.59	16.6 ± 1.1	<1	51.4 ± 4.4	71	23	32

These results show higher values for packed cell volumes, haemoglobins and red cell counts than many previous authors.

Van Loon and Clark (1943) used eighty-one adult mongrel dogs and took two hundred samples from these dogs. All were kennelled and fed standard rations. Blood was obtained from the femoral artery and the anticoagulant was ammonium and potassium oxalate. Haemoglobin was estimated using the Evelyn spectrophotometric method.

Their results are summarised below:-

R.B.C. per cu.m.m.	Haemoglobin grms/100 mls.	P.C.V. %	Reticulocytes %	W.B.C. per cu.m.m.
6,200,000	14.6	45.6	0 - 1.5	13,520

Results of the differential counts were:-

Neuts. %	Lymph. %	Mono. %	Eosin. %	Baso. %
71.8	20.1	3.7	4.4	rare

They stated that they found no variations due to age, sex or breed, but give no results.

Smith (1944) estimated the haemoglobin values from sixty normal dogs and found the variation to be 10-22 grms. per 100 mls. Using experimental dogs the physiological normal haemoglobin value was considered to be 18 grms. per 100 mls.

Also in 1944 Bloom and Meyer did work on the peripheral blood, as well as bone marrow studies. No age or breed was given for /

for the dogs used. These results add little new information as regards the peripheral blood picture. They noted that 0-3 normoblasts (average 0.6) occurred for every 100 white cells counted on a differential.

Haemoglobin (11-13.1 grms. per 100 mls.) was low considering the red cell results were relatively high - 5,960,000 - 7,440,000 per cu.m.m. (average 6,629,000 per cu.m.m.).

Total white counts varied considerably from 10 to 17.5 thousand per cu.m.m. (average 13,500 per cu.m.m.). No correlation between age, sex and breed was attempted, as these results were intended only as a comparison with the bone marrow picture.

Ederstrom and De Boer (1946) used one hundred and fifty-eight normal dogs aged from one day to adult. All were housed in experimental kennels and the adults were stray mongrels. Their results are given according to the animal's age, and are as follows:-

The erythrocyte counts average 3.61 million per cu.m.m. for one to three day old pups. The values declined until two weeks old and then rose slowly to the adult value of 6.08 million per cu.m.m. Dogs were considered adult at six months of age.

Haemoglobin and packed cell volumes followed a similar pattern. In pups up to three days of age the values were 10.5 grms. per 100 mls. and 39.8% respectively. These fell until three weeks of age and then rose slowly to adult values of 12.5 grms. per 100 mls. and 43.4%. Specific gravity ranged from 1.0468 (first three days of life) to 1.054 (adult value).

Corpuscular values were as follows:-

Age	M.C.V. cu.µ.	M.C.H. µµ. grms.	M.C.H.C. %
First three days of life	111.1	29.4	26.4
Adult	72.7	20.9	29.1

The haemoglobin values in this article appear to be very low. Haemoglobin was estimated using a Cenco-Sheard-Sanford photelometer. (Todd & Sanford 1939).

Allen, Sanderson, Milham, Kirschoen and Jacobson (1948) give normal values for dogs of unknown age or sex prior to irradiating them. The platelet counts averaged 300,000 per cu. m.m. and sedimentation rates 7.06 m.m. per hour. They also give results for red counts, haemoglobin, packed cell volume, white counts and differential counts, but added no new information to that already available.

Crafts (1948) gave values for forty-five samples from fourteen dogs (adult females weighing 8-12 kilograms), prior to experimentation. Red cell counts, haemoglobin, white cell counts and differential white counts were done, but again no new information was gained from these figures.

Also in 1948 El Hindaway published results from seventy-four dogs. These were strays or dogs obtained from the Cairo Veterinary School. They were housed in kennels while the work was /

was carried out. The ages ranged from birth to adult.

The results were presented as the normal values for Egyptian dogs.

Blood was collected from an ear vein. Haemoglobin was estimated using a Sahli-Leitz haemoglobinometer.

Platelets were counted by the method of Walker and Sweeney. The dogs were classified according to age. Pups were birth to three months of age, young dogs from three to nine months and adults over one year.

His results can be briefly summarised:-

	R.B.C. millions per cu.m.m.	Average Haemoglobin grms/100 mls.		Retic. %	W.B.C. thousands per cu.m.m.	Platelets per cu.m.m.
Pups	3.15-5.92	8.7		3.59	8.12	280,000
Young	3.55-6.77	8.5		1.33	11.06	286,000
Adults	3.28-8.7	Male 9.6	Female 10.1	1.2	12.76	320,000

The differential white cell counts showed that pups have a higher percentage of lymphocytes and a lower percentage of neutrophils than adult dogs.

Rekers and Coulter (1948) examined blood from ninety-one normal adult mongrels aged between nine and twenty-four months. All were housed in kennels, fed a standard diet,and wormed.

The /

The values for their results are given in the table.

	R.B.C. millions per cu.m.m.	Haemoglobin grms. per 100 mls.	Packed Cell Volume %	Sed. Rate m.m. per hour	Retic. %	Platelets thousands per per cu.m.m.
Range of Variation	4.5-8.8	11.2-19.5	40-70	0-25	0-2.7	0-420
Mean	6.22	14.78	51.3	3.3	0.78	227

Total white and differential white cell counts were as follows:-

	W.B.C. per cu.m.m.	Neuts. %	Lymph. %	Mono. %	Eosin. %	Baso. %	Blast forms %
Range of Variation	545-27,950	42-99	0-55	0-4	0-39	0-3	0-6
Mean	1o,582	75.11	16.82	0.15	6.88	0.14	0.9

No plasma cells were noted in the smears.

These results show enormous variations, especially in the total white counts and differential white count results. Many of these extremes would appear to be from pathological cases. No explanation is given for the wide variations in results.

Kudriawcew (1951) gives the following results (Russian) for normal dogs. No age, sex, or breed details are included.

	Average	Range of Variation
R.B.C. millions per cu.m.m.	7.2	6 - 8.5
Hb. %	93.1	84 - 108
Colour Index	1.1	0.8 - 1.4
Hb. Saturation µ.grm.	24	23 - 26
W.B.C. thousands per cu.m.m.	9.3	6.8 - 11.8
Platelets thousands per cu.m.m.	400	250 - 550
Basophil %	0.5	0 - 1
Eosinophil %	6	2.5 - 9.5
Stab. %	4	1 - 6
Segmented %	55	43 - 71
Lymphocyte %	25	17 - 32
Histiocyte %	6	4 - 8
Monocyte %	3.5	1 - 5
Specific Gravity	1.050	1.044 - 1.053

No new information is given, but these results are an interesting comparison of Russian work and that done in other countries.

Knowles (1955) in his paper on normal and abnormal values in blood considers the normal white cell count in the dog to /

to be 10,500 - 12,500 per cu.m.m. Sedimentation rates as 0 - 15 m.m. per hour, and packed cell volumes 50 - 55%. The corpuscular values are as follows:-

M.C.V. cμ. = 64 - 72

M.C.H. μμ.grms.= 19 - 23

M.C.H.C. % = 29.4 - 32.6

He gives no explanation for normal variations.

Afonsky (1955) used forty-three dogs obtained from three different sets of experimental kennels. The results from each set of kennels is listed but no age or breed details are given:-

In the article ranges of variation were also given. From these it is seen that there are considerable variations from one kennel to another. The author laid no stress on these facts. The average figures are shown here.

	R.B.C. millions per cu.m.m.	Hb. grms. per 100 mls.	P.C.V. %	M.C.V. cu μ.	M.C.H. μμ. grms.	M.C.H.C. %	W.B.C. thousands per cu.m.m.
Kennel 1	6.3	16.2	50	79	26	32	5.4-12.4
Kennel 2	6.1	14.7	50	83	25	30	-
Kennel 3	7.1	17.8	50	-	24	-	8.9-15.4

He noted variations between one dog and another and also in the same dog on different occasions. He found that haemoglobin /

haemoglobin values varied depending on the methods used. In this article he used the acid haematin and oxyhaemoglobin methods.

He found no variations due to sex. He considered, in the summary, that the normal picture for dogs had not yet been established.

Ugliaroro and Alder (1957) did five hundred and sixty-two bleedings from forty-four female beagles. They performed red cell counts and packed cell volume estimations on the samples, and considered it possible to correlate packed cell volumes and the erythrocyte count accurately.

Baer, Peck and McKinney (1957) used one hundred and twenty female mongrel dogs kept under physiological conditions. No age is given. The results show large variations which are probably due to both young and old dogs being used. For this reason the results are not of as much value as they might have been.

The erythrocyte count showed a 95% range of 4-8 million per cu.m.m. and the haemoglobin a 95% range of 13-2o grms. per 100 mls.

Haematocrit (Westergren) varied from 40-55% (89% of the dogs falling in the 40-50% range).

Sedimentation rates (Westergren) were usually nil but fourteen dogs showed variations of 10 m.m. per hour on various occasions.

The /

The 95% range for leucocytes counts was 5,000-15,000 per cu.m.m. Differential counts were badly described and variations were large.

Anderson and Gee (1958) sampled five hundred kennelled, normal Beagles. Their article is comprehensive and covers many aspects which have been inadequately dealt with before. It is impossible to give all their results but the more major aspects will be given.

The picture for new-born puppies was:-

Erythrocytes per cu.m.m. 5,750,000

Leucocytes per cu.m.m. 16,500

Values for immature dogs (six months) and mature dogs (three years and over) were as follows:-

	Immature		Mature	
	Male	Female	Male	Female
Erythrocytes millions per cu.m.m.	7.09	7.24	8.39	7.75
P.C.V. %	42.3	41.9	50.3	46.6
Haemoglobin grms/100 mls.	13.8	13.9	18.6	16.5
Sed. Rate m.m. per hour	11.7	5.4	1.0	3.7
Platelets thousands per cu.m.m.	252	306	407	486
Leucocytes per cu.m.m.	14,047	13,844	12,955	15,069

The absolute differential figures indicate that lymphocytes were higher, and neutrophils slightly lower in pups than in mature dogs.

Variations with age were considered fully. Red cell counts, haemoglobin and packed cell volumes fell until dogs were four weeks old, and then rose slowly to adult values. Leucocytes were highest at two months of age, and then fell slowly to the adult values. Variations in the types of white cell in the first year of life are given in full.

Sedimentation rates increased when the puppy's teeth were erupting. They found diurnal variations to occur in both red and white cell counts, (red cells fell up to ¾ of a million per cu.m.m. in the morning, and the white cell count rose by up to 2,000 per cu.m.m.).

They considered males to have higher red counts, haemoglobin and packed cell volumes than females.

They also described some variations occurring in pregnancy. These will be dealt with under the appropriate sections.

Irfan (1958) in his Ph.D. thesis gives considerable data on the blood picture of normal dogs. The age, breed and sex of each dog is given as are all the results for each dog. He used one hundred and sixty-five dogs, of which seventy came from two separate research kennels, and the rest were pets. Ages varied from one month to fifteen years. Twenty-one breeds were studied but /

but only four were done in large numbers. These were Beagles, Greyhounds, Mongrels and Spaniels.

These four breeds are each considered separately. He found greyhounds and possibly other hounds had higher red cell counts, packed cell volumes and haemoglobins than other breeds. He found no sex variations but definite variations with age. He described the characteristic drumstick on the female neutrophil, and the staining of eosinophils. He did not describe the vacuolations seen in greyhound eosinophils.

He derived a standard blood picture for dogs (not including greyhounds). Briefly his results were as follows:-

	1 month	4 months	6 months	Adult
Red cell count millions per cu.m.m.	3.7	5.19	5.6	6.11 ± 1.05
Haemoglobin grms/100 mls.	11.3	15.0	16.0	17.5 ± 1.5
P.C.V. %	32.6	45.7	49.5	52 ± 6

Red blood cells, haemoglobin and packed cell volumes rose steeply until six months of age and then slowly from six months to one year.

Corpuscular Values (no age variations)

M.C.V. cu.μ. = 86 ± 13
M.C.H. μμ. grms.= 30 ± 4
M.C.H.C. % = 33 ± 5

White /

White Cell Picture per cu.m.m.

Dogs under 2 years	14,000 ± 2,500
Dogs over 2 years	10,500 ± 3,000
All dogs	11,500 ± 3,500

Lymphocytes were high until two years of age and after this they decreased.

Sedimentation Rates (all ages)

1 hour	2 hours	3 hours	24 hours
0.8 m.m.	1.8 m.m.	3.5 m.m.	8.1 m.m.

Differential White Counts

	Up to 2 years of age	Over 2 years of age
Neutrophils	7,230	7,734
Juvenile	15 ± 4	20 ± 8
Stab.	980 ± 770	1,032 ± 680
Seg.	6,300 ± 1,400	6,615 ± 1,050
Lymph.	5,250 ± 1,470	1,943 ± 787
Mono.	478 ± 300	401 ± 260
Eosin.	1,162 ± 900	582 ± 500

Sex /

Sex variations

Age	Male Hb. grms.per 100 mls.	R.B.C. millions per cu.m.m.	P.C.V. %	Female Hb. grms. per 100 mls.	R.B.C. millions per cu.m.m.	P.C.V. %
1 month	11.6	3.8	35.5	11.2	3.72	32
2 months	11.7	4.87	34.2	11.7	4.06	33.2
4 months	15.2	5.39	46.4	15	5.1	45.4
6 months	15.4	5.82	50	16.6	5.5	49
9 months	15.9	5.74	54	18.1	6.02	51
1 year or over	18	6.44	53.8	19.2	6.24	54.4

He found no significant variations due to sex.

Blood picture of Various Breeds (over one year of age)

	Beagle	Greyhound	Spaniel	Mongrel	Mixed Breeds
Hb. grms. per 100 mls.	18	21.4	16.2	17.25	17.7
R.B.C. millions per cu.m.m.	5.95	7.36	6.22	6.11	6.38
P.C.V. %	51.6	63.8	52.5	50.5	54.8
W.B.C. per cu.m.m.	15404	10112	11207	10812	10854
M.C.V. cu.μ.	88.5	87.6	80.03	84.1	87.4
M.C.H. μμ. grms.	30	30.09	28.4	29.1	28.3
M.C.H.C. %	33.8	34.4	32.6	34.1	32.1

He concluded that beagles had higher white cell counts than other dogs but made no attempt to compare beagles from one kennel with those from another.

Further reference will be made to this thesis later on, under the appropriate sections. Irfan did indicate that the results of other workers depend on the methods and techniques used.

Carr and Schloerb (1959) did packed cell volume estimations on ninety-one dogs. They used Wintrobe tubes spun at 3,000 r.p.m. for thirty minutes. Their mean results and range of variation were 45.4% (59-34%).

Didisheim, Hattori and Lewis (1959) did ten determinations on six dogs. Packed cell volumes averaged 47% and sedimentation rates 5 m.m. per hour. Differential white counts showed stabs as 8.6%. No absolute figures or total white counts were given, so differential results mean very little.

Custer (1961) considers that the normal range of white cell counts in dogs is 8-14,000 per cu.m.m. A figure below 8,000 per cu.m.m. is only normal in old dogs, and a figure above 14,000 per cu.m.m. indicates infection.

Schalm (1961) gives results for normal dogs. These were usually compiled from results of many previous workers, all of whom have been discussed in this review. He gives his own results for twenty normal bitches presented for spey operations. Ages but no breeds are given.

Purushotham and Mahendar (1963) gave results for blood values from six normal dogs. No age, sex or breed was given and the results added little of value to the existing literature.

The /

The sedimentation rates were all above three m.m. per hour. No total white cell counts are given but the differential white count is given in percentages, and as a result are valueless. The only feature of interest was that they gave the clotting times for each dog, as follows:

	clotting time in minutes		clotting time in minutes
Dog 1	2.5	Dog 4	2.5
Dog 2	3	Dog 5	3
Dog 3	2.5	Dog 6	2.5

Blakemore (1963) wrote three articles on clinical haematology and in the course of these gave the following values for normal dogs. No specific details concerning the dogs were included.

Packed cell volumes - Average 42% Range 37 - 58%

Sedimentation rates - 0 - 25 m.m. per hour

Total white cell and differential white cell counts:

W.B.C. thousands per cu.m.m.	Neut. %	Lymph. %	Eosin. %	Mono. %	Baso. %
6 - 14	60-70	15-30	1-15	1-6	0-1

Canine Medicine, edited by Hoskins, Lacroix and Mayer (1953) gives a long table showing the blood pictures given by various authors. The detailed work of these authors has been dealt with in the review.

Albritten in "Standard Values in Blood" (1955) gives data in the form of tables on the blood picture of both man and various animals. No ages are given.

Wintrobe /

Wintrobe (1956) in "Clinical Haematology" lists the work of authors already reviewed here.

Benjamin (1962) lists normal blood values for the dog. All the authors listed have been reviewed here.

Boddie (1962) in his text book gives the figures of Mayerson. Dukes (1955) gives those of Mayerson and Albritten.

In Current Veterinary Therapy, edited by Kirk (1964-5) a list of normal blood values for the dog is given. The main ones are as follows:-

	Range	Average
R.B.C. (millions per cu.m.m.)	5.5 - 8.5	6.8
Hb. (gms/100 mls.)	12 - 18	14.9
P.C.V. (%)	37 - 55	45.5
M.C.V. (cu.µ.)	67 - 77	69.8
M.C.H. (µµ. gms.)	19.5 - 24.5	22.8
M.C.H.C.(%)	31 - 34	33
Platelets (per cu.m.m.)	2-9 x 10^5	4.7
Fragility - minimum	0.40 - 0.50	0.46
maximum	0.32 - 0.42	0.33
W.B.C. (per cu.m.m.)	6 - 18,000	11,000
Band Neut. (%)	0 - 3	0.8
Neutrophil (%)	60 - 77	70
Lymphocyte (%)	12 - 30	20
Monocyte (%)	3 - 10	5.2
Eosinophil (%)	2 - 10	4
Basophil (%)	Rare	Rare

Also given are absolute values for white blood cells from dogs classed in age groups.

Even in so recent a publication no mention is made of the reasons for such large ranges of variation, e.g. W.B.C. 6 - 18,000 per cu.m.m.

DISCUSSION OF THE LITERATURE REVIEWED

A considerable amount of work covering over one hundred years, has been reviewed, and it may be thought that further work is unnecessary.

Despite the large number of results obtained durigg this period, I still do not consider that an adequate, overall picture, of the variations occurring in the peripheral blood of normal dogs, has been given. A breakdown of the literature reviewed will show this.

During the first period reviewed, prior to 1910, the maximum number of dogs used in a survey was twenty (Busch and Van Bergen, 1902). This is surely an inadequate number. Most of the twelve authors reviewed gave results for red cell counts, haemoglobin, total white cell counts and differential white cell counts, but no other estimations were performed. Only one article (Burnett and Traum, 1905) correlated the blood picture with age and one (Goodall, 1910), mentioned that feeding and environment affected the blood picture but gave no results to confirm this statement. The age of the dogs used is given in two articles and the sex in three. Little attempt was made to correlate these facts with the blood picture, and no author described changes due to management or normal physiological stress.

In the period 1910 - 1940 the work done was more comprehensive /

comprehensive but still left a lot to be desired. The number of dogs studied in the surveys rose. Six authors used thirty or more dogs and two authors used groups of over sixty dogs. The estimation of corpuscular values was introduced by five authors, reticulocyte counts by two and platelet estimations appeared in three articles. Packed cell volume estimations, specific gravity, and red cell fragilities were also dealt with during this period.

Variations in the blood picture due to age were dealt with especially by Landsberg, although even he did not use serial bleedings to see what changes occurred in any particular dog as it aged. Variations due to sex were covered by Leichsenring et al (1932) and Landsberg (1939 and 1942).

Changes occurring after pregnancy are dealt with briefly by Wells and Sutton (1915).

All the dogs examined during this period were kennelled and fed on standard diets. No pet dogs were used.

Of the sixteen articles reviewed nine omitted to give the dog's age and six the sex. In all cases the breeds were given as "mongrel" or omitted completely. Nobody tried to correlate the blood picture with all these three facts. No author described changes due to management and no specific work on any form of physiological stress in normal dogs was done.

Up to the end of 1940 all the non-biochemical examinations associated with the cellular contents of the blood had been dealt with, but not one author did all the estimations in one paper.

Since /

Since 1940 a lot more work has been completed, presumably to try and fill the gaps in the information already available. However, of the twenty-three articles reviewed eleven of them give no age and thirteen omitted to mention the breed of dog used. Such work contributed very little to the existing information. Six authors, Morris et al (1940); Mundhenk (1941); Mulligan (1941A); Ederstrom and De Boer (1946); El Hindaway (1948) and Rekers and Coulter (1948) gave the breed of dog used as "mongrel" and also gave indications of the age and sex. Only two authors, Anderson and Gee (1958) and Irfan (1958) give age, sex and exact details of the breeds of dogs involved ⊖ these authors are in fact the only ones in this review to have mentioned all these facts exactly. These eight articles are the only ones which can be considered to have dealt with the subject of normal variations in the blood picture of the dog with any degree of exactitude in the past hundred years.

Of these eight articles only four, Morris et al (1940); Mulligan (1941A); Mundhenk (1941); and Irfan (1958) dealt with dogs which were not permanently housed in experimental kennels. Results from dogs which are permanently kennelled cannot be considered as truly representative of the normal dog population.

Only one ofthe remaining articles, Irfan (1958) gave the individual results from dogs which lived under different systems of management, but he made no attempt to compare the results obtained from dogs under one system with those from another.

From /

From this survey of the literature it is quite clearly seen that no single author has carried out a thorough investigation of the variations in the normal blood picture in the dog, although several authors have dealt with one or two specific aspects of the problem. Not one author has dealt fully with the changes associated with oestrus, false pregnancy, different systems of management, the effect of travelling, lack of water, or the blood picture of dogs which work for a living. The effect of pregnancy, parturition, feeding, and exercise has been briefly considered but in no cases were any controls used for these studies.

For these reasons it was considered important to carry out the present work.

PRESENT WORK ON THE BLOOD PICTURE OF NORMAL DOGS

DEFINITION OF A NORMAL DOG

In much of the previous work the dogs used have been kennelled, fed on a standard diet and all were wormed and free of parasites. These were dogs used for experimental work and lived in a man-made environment. Pets or dogs living free get considerably more exercise and are exposed to the stresses of life to a much greater extent. These are the dogs which the veterinary clinician sees in his everyday work and therefore this type of dog was studied whenever possible.

For this work a normal dog was considered to be one which appeared clinically normal. It was in good bodily condition, had a normal temperature (101 - 102°F), normal pulse and respiration, was alert and active and showed no evidence of minor ailments such as otitis externa or skin lesions of any sort. A history of good health for at least a month prior to sampling was essential and in the majority of cases evidence was obtained to prove that the dog had remained clinically normal for at least a month after sampling.

The only exception to this rule were old dogs of eight or more years of age. A slight degree of otitis externa or conditions such as an interdigital cyst or slight clear occular discharge was considered to be normal in dogs of this age and associated with senility and not with any pathological condition. Such changes were /

were seen mainly in Spaniels and Labradors. Minor skin lesions were seen in many of the dogs from the largest kennels (I) but as the majority of dogs were affected this was unavoidable.

Provided the conditions stated above appertained, the environment from which the dog came was not considered important, nor was the type of feeding the dog was receiving. These facts were obtained, however, from the owner before sampling was commenced.

Faeces samples were obtained and worm egg counts performed on them. If these egg counts showed that the dog had a pathological worm burden it was not used in this work. Many of the younger dogs used had a small number of ascarids present, but no attempt was made to remove the parasites - they were considered to be so common in the dog population as to be normal.

ERRORS OF THE TECHNIQUES USED

Review of the Literature

Numerous authors have pointed out the large errors which are almost unavoidable when performing blood counts. Berkson, Magrath and Hurn (1940) estimated the error for red cell counts as 7.8% and for the white cell counts as 10.7%.

Macfarlane (1945) using the Haldane-Gowers technique for estimations of haemoglobin obtained an average error of ± 4.8%.

Biggs and Macmillan (1948A) obtained the following minimum errors in their estimations:- Haemoglobin (Haldane) 2-3%; red cell counts 7.8%; packed cell volume (micro method) 0.5%; and platelets 10-15%. Errors for corpuscular values varied from 2-9%. Biggs and Macmillan (1948B) considered the standard error for red cell counts to be 8-10%.

Biggs (1948) considered that the error in reticulocyte counts is often exaggerated by personal error; e.g. including or excluding cells to make the answer fit what you think it ought to be.

Many text books also comment on the large errors occurring in counting techniques, Dacie (1950) p. 17; Schalm (1961) p. 71-73; Darmady and Davenport (1958).

Present Work on Errors in Techniques

In the light of these findings twelve counts were performed on a single blood sample to estimate my own errors in these techniques.

Red /

Red cell counts:

Twelve counts were performed using twelve pipettes, and six double-sided chambers were filled. The pipettes were filled one after the other from one blood sample. Five large squares (i.e. eighty small squares) were counted each time. The mean result of the twelve counts was 6,320,000 per cu.m.m. - the highest 6,590,000 per cu.m.m. and the lowest 6,080,000 per cu.m.m. The error was 8.07% (± 4.035%). If the mean result of the two counts from each chamber was taken the error was 5.6% (± 2.8%).

White cell counts:

Twelve counts were performed in the same way as for the red cell counts. The mean result was 5,558 per cu.m.m. - the highest 5,850 per cu.m.m. and the lowest 5,200 per cu.m.m. This gave an error of 11.7% (± 5.85%). If the mean of the two counts from each chamber were taken the error was 8.9% (± 4.45%).

Platelet counts:

These were performed in the same way as the red and white cell counts. The total error was 10.3% (± 5.15%). The error for the mean of two counts from each chamber was 2.9% (± 1.45%).

Packed cell volume:

The total error for twelve estimations was 1.9% (± 0.95%).

Haemoglobin:

The total error for the twelve estimations was 1.5% (± 0.75%).

All /

All these estimations of errors in technique were performed before the work started and also while it was in progress. The second time the errors were slightly lower than those given here, as the author had become more adept in the use of the techniques involved.

Conclusions

As the errors were quite considerable it was decided to perform all the routine erythrocyte, leucocyte and platelet counts in duplicate, using two separate pipettes for each count and filling both sides of the counting chamber. If the two results differed by more than $\pm$ 5% they were repeated, in duplicate. In most cases it was not necessary to repeat the counts but occasionally, especially when a lot of work had to be completed in a short time, repeat counts were needed. The mean of the two counts was taken as the final result. By using these methods it was hoped that the errors would be reduced as much as possible.

Packed cell volumes and haemoglobin estimations were not performed in duplicate as the error was reasonably small.

ABBREVIATIONS

As a large number of the tables included in the results are of considerable length, it was found necessary to abbreviate some of the headings, in order to fit the tables into the space available. A key to the abbreviations used is given in alphabetical order below.

Band	Band form neutrophils
Baso	Basophils
Blast	Blast Cells, e.g. Lymphoblasts
Eosin	Eosinophils
Hb.	Haemoglobin
Lymph	Lymphocytes
M.C.H.	Mean Cell Haemoglobin
M.C.H.C.	Mean Cell Haemoglobin Concentration
M.C.V.	Mean Cell Volume
Meta	Metamyelocytes
Misc. cells	Miscellaneous Cells
Mono	Monocytes
Myelo	Myelocytes
Neuts.	Neutrophils
Norm	Normoblasts
P.C.V.	Packed Cell Volume
Plate	Platelets
R.B.C.	Red Blood Cells
Retic.	Reticulocytes
Retic. Endo.	Reticulo-endothelial Cells
S.G.	Specific Gravity
Sed. Rate	Sedimentation Rates
Seg.	Segmental Neutrophils
Stab.	Stab Neutrophils
Türk	Türk's Irritation Forms (proplasmocytes)
W.B.C.	White Blood Cells

SOURCES OF NORMAL DOGS

In order that variations in the blood picture due to breed, or managemental factors could be assessed, dogs were obtained in groups from several different sources. A description of the premises, methods of management and feeding for each separate group is given here. The number, ages, and breeds of dogs obtained from each source will be given in the Appendix.

(1) RESEARCH KENNELS (I) (Beagles)

The dogs obtained here were all beagles of varying ages. The kennels belonged to a very large pharmaceutical concern.

Management:

This was a self-contained breeding unit. Breeding bitches were housed in pens approximately 8 ft. x 5 ft., each pen being divided from the next by a wall 6 ft. high. Runs were attached to each pen and the dogs exercised there all day. Beds were raised six inches from the floor.

Bitches whelped in a specially heated room, again divided into pens, and stayed there after whelping for a period of three to ten days. After this, bitch and litter were moved into special rearing pens and remained there until the pups were six weeks of age.

Puppies were then transferred to the puppy house where they remained till four months of age. This house was large, divided

into /

into pens by low wire fences, and the bedding was sawdust. Adequate ventilation kept the house at a steady temperature. Up to 300 puppies were in this house at one time.

At the age of four months pups were moved out into houses having large runs attached to them. Some ten to twenty dogs lived in each house.

At the age of six months dogs were either added to the breeding stock or transferred to another establishment.

Feeding:

Pups were weaned at six weeks of age on to cooked meat, milk, and puppy meal. The meal was later replaced by special pellet food. At six months of age the dogs were getting two meals daily. Cod liver oil was given twice weekly and water was available ad lib. Adults received a diet of meat and pellet meal, and again water was always available. On a second visit the watering was carried out at less frequent intervals.

Other Aspects:

All puppies were vaccinated at three and nine weeks of age against distemper and hepatitis. No distemper is known to exist in the unit, but hepatitis is present and would occur if vaccination ceased for any reason.

Demodectic mange was present on a large number of dogs but only those showing considerable bald areas were treated.

Ascarids are present in all puppies despite routine de-worming being carried out every month. Most of the puppies used /

used had a faecal egg count of 1,000 - 2,500 eggs per grm. (Toxascaris leonina).

These premises were clean and very well run, but the buildings were old (mainly converted farm buildings). Up to 1,000 dogs were present in the kennels at any one time. Only members of the firm's staff were allowed to enter the buildings.

(2) RESEARCH KENNELS (S)

These kennels, which housed up to thirty-five dogs, were modern and specially constructed for the purpose. They belonged to a pharmaceutical firm in Edinburgh.

Each kennel housed one or two dogs and was divided from the next by a solid partition. Large runs were attached to each kennel in which the dogs exercised all day. Concrete floors were used throughout.

Adults, whelping bitches and puppies were all housed in the same building, although, naturally, each had a separate kennel. Air conditioning kept the temperature constant at all times.

This unit consisted of breeding bitches (beagles) together with other dogs used in the work of the Company. Latterly only beagles were kept. Only the kennel staff and research staff were allowed into the kennels.

Feeding:

Pups: After weaning are fed twice daily on puppy meal, milk and tinned dog food. Water was always available.

Adults /

Adults: Fed once daily on cooked meat, or tinned meat occasionally, and meal. Water was also always available although some dogs turned the water bowls over and were therefore without water during the night.

Other Aspects:

All dogs were vaccinated against distemper and hepatitis at nine weeks of age. No clinical case of distemper has ever occurred in these kennels. Demodectic mange occurred sporadically and was always treated as soon as it appeared. Routine de-worming was carried out and helminth infestations were light, most puppies having 200 - 500 eggs per grm. of faeces. (*Toxascaris leonina* and *Toxocara canis*). These kennels are clean, well ventilated and well managed.

(3) COLLEGE KENNELS

The dogs obtained from these kennels belonged to College Departments. The kennels were rather old but were modernised during the course of this work.

Management and Feeding:

Each dog was housed in a separate kennel measuring 4 ft. x 3 ft. Bedding was on newspaper laid over wooden boards. Exercise was taken in a communal run. Each dog was fed once daily on cooked meat and meal. Water was available several times daily.

Animals suffering from non-infectious diseases were also housed in these kennels but not used for this work. Only College staff and students were allowed into the kennels.

(4) /

(4) BOARDING and BREEDING KENNELS (H)

These kennels were privately owned and the dogs housed there were either breeding animals belonging to the owner, or animals being boarded there during the holiday period. Very few boarders were sampled owing to the difficulty of obtaining the owners' permission.

The buildings were rather old and consequently not as efficiently ventilated and heated as in more modern premises. The temperature, however, was reasonably stable.

Each dog was housed in kennels 4 ft. x 3 ft. arranged in two tiers one above the other. The bedding used was straw, which made the kennels untidy. Dogs of all breeds and ages were housed in the same building, but in separate kennels. Whelping bitches were housed separately, although when space was not available they whelped in the main kennel block.

Exercise was in a communal concrete run and taken three times daily. The dogs belonging to the kennel owner were allowed to run free in the large garden in fine weather. The kennels were in a town and visitors were frequent.

Feeding:

Pups: Weaned at six weeks of age and then fed milk, meat and puppy meal.

Adults: Fed once daily on cooked meat and biscuits, or sometimes bread. Water was only available after feeding or while the dogs were out in the exercise yard. No water was available at night.

Other /

Other Aspects:

All the kennel owner's dogs were vaccinated against distemper and hepatitis and leptospirosis, but de-worming was not carried out unless clinical signs of worms were seen.

(5) PRIVATE KENNELS (C) (Greyhounds)

With the exception of one spaniel all the dogs houses here were greyhounds. The kennels werw wooden and wire mesh structures measuring 6 ft. x 6 ft. with a raised wooden bed some two feet from the ground on one side. The bedding material was straw. These kennels were situated in old farm buildings with the exception of three kennels in a wooden hut. The floors in the farm buildings were rough and uneven and the walls white-washed stone. Dirt collected easily despite frequent cleaning. Ventilation and lighting were moderate. The buildings were in the country well away from other houses and visitors were infrequent.

Feeding:

This was excellent. Large quantities of meat, biscuit meal, vegetables and bread were fed, together with milk and eggs. Large bones were given twice weekly. Feeding occurred once daily and the dogs ate until satisfied. Water was available after feeding and exercise.

Exercise:

This was extensive, each adult dog being walked three to four miles daily as well as receiving exercise in a large field. Many of the dogs were also being raced at regular intervals.

Other /

Other Aspects:

All dogs were vaccinated against distemper, hepatitis and leptospirosis, and routine de-worming was carried out on the puppies up to six months of age. Ascarid eggs, however, appeared regularly in the faeces samples examined, although counts were never above 750 eggs per gramme of faeces.

(6z) PETS

The dogs in this group were mostly living as household pets in the City of Edinburgh. Management, feeding and amount of exercise varied considerably. If any variations in the blood picture of dogs in this group are significant the precise conditions of management will be discussed in the appropriate section.

Two main groups of dogs classed as pets vary slightly from the others. One group are dogs of varying breeds, living as household pets some fourteen miles out of the city, in village communities. The second group are greyhounds living in sheds, either in the owner's garden, or on railway property away from the owner's house. The number of dogs in each of these groups will be shown in detail in the Appendix. (when applicable).

(7) WORKING DOGS

These dogs all lived in the country and were mainly gun-dogs. Some were privately owned and worked occasionally, and a few belonged to gamekeepers or farmers and were out working every day of the year. The method of management varied but was generally good. Where details of management or amount of work done are required they will be given under the appropriate section. Greyhounds are not included in this group.

For /

For identification purposes in this work each of the sources was given a code letter. These letters are as follows:-

	Order given in the text	Code Letter
Beagles from research kennels	(1)	I.
Dogs from research kennels	(2)	S.
Dogs from College kennels	(3)	R.D.V.C.
Boarding and Breeding kennels	(4)	H.
Private kennels (Greyhounds)	(5)	C.
Pets	(6)	P.
Working Dogs	(7)	W.

Each of the dogs from any particular source were given a number, e.g. the sixth dog bled from source S. was given the code S.6. If a dog was bled more than once it was given a further letter of identification. For the second bleeding the letter 'a' was added to the code, for the third bleeding the letter 'b' was given, and so on. For example, the fourth bleeding from the sixth dog from source S. would be S.6c., or the sixth bleeding from the tenth dog obtained from kennel H would be H.10e.

These code letters and numbers will be given so that each dog can be easily identified.

Tables showing the number of dogs in each kennel is given in the Appendix pages 1-8. Code numbers and details for dogs specifically mentioned in the text are also given. Details of the breeds of dogs used in the work are shown in the Appendix pages 9-10.

PRESENT WORK ON THE BLOOD PICTURE OF NORMAL DOGS

Parts of this work are designed to be read in conjunction with the tables of results which are presented in the Appendix.

HOURLY, DAILY AND WEEKLY VARIATIONS IN THE BLOOD PICTURE OF NORMAL DOGS

It was considered desirable to find out to what extent the blood picture of healthy dogs varies from day to day and week to week. This was done so that if any variations were seen to occur in such conditions as pregnancy, it would be possible to tell if these variations were in fact due to pregnancy or just to normal weekly variations.

REVIEW OF THE LITERATURE

Stebbins and Leake (1927) showed that diurnal variations occurred in the specific gravity of normal dogs' blood. They used six dogs and obtained the following differences between the maximum and minimum results:

Dog 1.	Dog 2.	Dog 3.	Dog 4.	Dog 5.	Dog 6.
0.0024	0.0068	0.0031	0.0045	0.0062	0.0036

Mundhenk (1941) gives the results obtained from three dogs, bled at two hourly intervals, for a period of twenty-four hours. He found that considerable variations occurred. The extremes of variation he found over twenty-four hours are as follows:

Dog 1.		Dog 2.		Dog 3.	
R.B.C. millions per cu.m.m.	W.B.C. thousands per cu.m.m.	R.B.C. millions per cu.m.m.	W.B.C. thousands per cu.m.m.	R.B.C. millions per cu.m.m.	W.B.C. thousands per cu.m.m.
4 - 7	6 - 13	5 - 7	$2\frac{1}{2}$ - $5\frac{1}{2}$	5 - 8	10 - 15

Such /

Such large variations could interfere with the interpretation of results obtained from diseased animals. The maximum variations between one bleeding and the next were red cells 500,000 per cu.m.m. and white cells 1,000 per cu.m.m.

Afonsky (1955) considered that variations in the blood picture occurred from the same dog when it was sampled several times. He found the following daily variations:

P.C.V.	±	2.3%
Haemglobin	±	0.96 grms/100 mls.
R.B.C.	±	0.66 millions per cu.m.m.

All these authors found daily variations in the blood picture but the extent of the variations differed. The present work will try and establish the extent of the variations seen in the types of dogs used in this work.

PRESENT WORK

Only the main estimations are considered. If the total white cell counts fluctuate then it can be assumed that the differential counts will also vary.

The results have been obtained from dogs used as controls for the various experiments performed during the work on normal dogs.

A total of six dogs, from which nineteen samples were taken, were used to establish hourly variations. The code numbers of all these dogs are given in the results.

Four /

Four dogs were used to establish variations between one day and the next. Twenty-six samples were obtained.

Four dogs were sampled at weekly intervals for several weeks, thirty-seven samples being removed, to find out the variations occurring from week to week. Exactly the same technique was used to examine each sample from each dog, thereby making the technique error as near the same for each dog as possible.

RESULTS

HOURLY VARIATIONS

Four dogs, R.D.V.C.2, R.D.V.C.3, S.23 and H.25 were used as controls and sampled before and after experiments on the effect of travelling and exercise. The tables, showing the variations which occurred between the first and second samples, are given in the Appendix page 11.

Two other dogs, R.D.V.C.2 and S.23, were sampled at hourly intervals. The tables given in the Appendix page 12 show the variations between each sample.

The full results for all six dogs are given in the Appendix pages 13 and 14.

If the results from all six dogs are considered, the average of the variations between one sample and the next is as follows. The standard deviation from this average is also given.

R.B.C. millions per cu.m.m.	0.134 ± 0.161
P.C.V. %	0.46 ± 0.50
W.B.C. thousands per cu.m.m.	0.463 ± 0.272
Platelets thousands per cu.m.m.	19.8 ± 11.4

DAILY VARIATIONS

Four dogs, S.24, R.D.V.C.1, P.77 and S.23 were used as controls for bitches in oestrus. All the samples were taken at the same time in the morning at intervals of two or three days. The variations between one sample and the next shown by these dogs is given in the Appendix, pages 15 - 16.

The average variation between one sample and the next from all four dogs, together with the standard deviations from this average, are as follows:-

R.B.C. millions per cu.m.m.	0.258	±	0.192
P.C.V. %	1.47	±	1.14
W.B.C. thousands per cu.m.m.	1.786	±	1.485
Platelets thousands per cu.m.m.	27.7	±	21.4

Although considerable variations occur from day to day the values fluctuate up and down and do not show a steady trend in one direction all the time. If the values did show a consistent trend in one direction only, the difference between the first and last sample in the series would be considerable. This is not the case. The differences between the maximum and minimum values obtained from each dog are shown in the Appendix, page 17, and illustrate this clearly. The number of days during which sampling took place is also shown.

The average difference between the maximum and minimum values obtained during the period of sampling, from all four dogs, are as follows. The standard deviations are also given.

R.B.C. millions per cu.m.m.	0.657	±	0.228
P.C.V. %	3.3	±	0.90
W.B.C. thousands per cu.m.m.	4.162	±	1.856
Platelets thousands per cu.m.m.	67	±	11.4

The full results for each dog are given in the Appendix, pages 18 and 19.

WEEKLY VARIATIONS

Four dogs S.2, H.31, H.19 and H.40 were used as pregnancy controls and these results are presented here. The samples were taken at the same time each morning and at weekly intervals. The full results for each dog are in the Appendix, pages 20 and 21.

The variations in the values obtained from week to week from each dog are given in the Appendix, pages 22 and 23.

The average variation between samples, from all four dogs, is given in the following table together with the standard deviations.

R.B.C. millions per cu.m.m.	0.450 ± 0.260
P.C.V. %	2.15 ± 1.7
W.B.C. thousands per cu.m.m.	1.011 ± 0.650
Platelets thousands per cu.m.m.	25.8 ± 14.8

The weekly variations showed an up and down fluctuation but like the daily variations did not show a continuous trend in one direction. As a result the differences between the maximum and minimum values obtained from each dog was not very large. This is shown in the table, Appendix, page 24. The period during which sampling took place is shown also.

The average difference between the maximum and minimum results, from the four dogs, together with the standard deviations are as follows:-

R.B.C. millions per cu.m.m.	1.156 ± 0.203
P.C.V. %	6.0 ± 3.0
W.B.C. thousands per cu.m.m.	3.523 ± 1.145
Platelets thousands per cu.m.m.	86 ± 11.8

DISCUSSION OF RESULTS

It has been clearly shown that variations do occur in the blood picture without any pathological abnormality being present.

The hourly and half-hourly variations are not very large, but do occur. If the average variation is taken and twice the standard deviation added to it, then the following results are obtained.

R.B.C. millions per cu.m.m.	0.456
P.C.V. %	1.46
W.B.C. thousands per cu.m.m.	1.007
Platelets thousands per cu.m.m.	42.6

If the blood picture varies by the same or more than these figures, when hourly or half-hourly samples are taken, then the dog is probably abnormal. It would be wise to check by taking a further sample an hour later to see if the trend continues.

In a similar way if twice the standard deviation is added to the average variation seen from samples taken at intervals of two or three days, the following results are obtained.

R.B.C. millions per cu.m.m.	0.642
P.C.V. %	3.75
W.B.C. thousands per cu.m.m.	4.756
Platelets thousands per cu.m.m.	70.5

In the results presented, the white cell counts slightly exceeded the figure given here on two occasions. As white cell counts are capable of rapid fluctuations it would again be prudent

to /

to check a borderline variation, by taking another sample, before stating that the dog was, in fact, abnormal.

The variations which occur in the normal dog's blood picture are cyclic. There are small variations from hour to hour during any one day, the values going up and down but not progressing in one direction all the time. There are also variations from day to day which are larger than the hourly variations especially with regard to total white cell and platelet counts. The white cell counts can vary by up to 4,800 per cu.m.m. and the platelet counts up to 70,000 per cu.m.m. from day to day, without any abnormality being present. These variations rise and fall but again do not progress rapidly in one direction for very long. This is clearly seen from the results. The total white count may vary by up to 4,000 per cu.m.m. from day to day but the largest difference seen between the maximum and minimum white cell counts from a dog sampled over a period of fifteen days was only 6,350 per cu.m.m.

The last group of variations examined in this work are those from week to week. These variations behave in exactly the same way as do hourly or daily variations, fluctuating up and down. If twice the standard deviation is added to the average weekly variations we find that the red cell counts can fluctuate by up to 970,000 per cu.m.m., the white cells up to 2,311 per cu.m.m. and the platelets up to 56,000 per cu.m.m. from week to week. The largest difference seen between the maximum and minimum values obtained from dogs bled over periods of eight to ten weeks were: red cells 1,243,000 per cu.m.m., white cells 5,000 per cu.m.m. and platelets 102,000 per cu.m.m. Each dog's values therefore fluctuate /

fluctuate only between set limits and do not go outside these limits unless some abnormality causes them to do so.

The reason for the weekly variations in white cell counts and platelet counts being lower than the daily variations is possibly due to the individual variations of the dogs used, as well as environmental influences. The environmental influences are considerable and will be discussed later.

Each dog has its own individual blood values, some values being low and some high. For example, two dogs A and B may have normal white cell counts of 8,000 per cu.m.m. and 15,000 per cu.m.m. respestively, and if a week later A has risen to 10,000 per cu.m.m. and B to 17,000 per cu.m.m. there is no reason to suspect any abnormality. If, however, A has risen to 17,000 per cu.m.m. some abnormality would be suspected. Each normal dog's values fluctuate, daily or weekly, between set limits. This does not mean, however, that each dog has the same limits.

When considering the daily or weekly fluctuations of a normal dog's blood picture, it is not the absolute values which one should concentrate on, but the amount of variation between the first sample and the second.

In a normal healthy dog, the blood values should show fluctuations from hour to hour, day to day, or week to week, which are less than the values given in the discussion. If the fluctuations /

fluctuations are greater than these values, or progress in one direction only, some abnormality should be suspected, especially if a further sample confirms the first results.

The results presented agree with those of Afonsky (1955) and in principle with those of Mundhenk (1941). The extremes of variation shown by Mundhenk are larger than the values obtained in this work.

VARIATIONS DUE TO SEX

In this section of the work dogs from two separate kennels were used. In these kennels there were enough dogs of both sexes to obtain a reasonable comparison. Two sets of kennels were used to ensure that if any variations occurred in one set of results these could be checked against the results from another source. In this way it was hoped that variations due to factors other than sex could be ruled out.

METHOD

All the dogs from Kennel I were beagles, living on the same premises and having the same type of management. The method of management of young puppies and adult dogs of course varies, but variations due to these differences in management were eliminated by dividing the dogs into age groups. By this means any variations due to sex could be detected, as results from male and female dogs living in identical conditions and of the same breed and same age were being compared.

Dogs from Kennel C were treated in an identical manner, only in this case all the dogs used were grey hounds. Bitches who had just whelped, were suckling a litter or had just finished suckling a litter, were not used in this work.

RESULTS AND DISCUSSION /

RESULTS AND DISCUSSION

Kennel I (Beagles)

A total of forty-seven dogs were used of which twenty were males and twenty-seven females. The ages ranged from three months to four years. Many of the adult bitches had had one or more litters but if this is of special significance the exact details for each bitch will be given. The reasons for the omission of results from dogs aged six months to one year are given in the section dealing with water deprivation.

Kennel C (Greyhounds)

A total of forty-nine dogs were used of which thirty-two were males and seventeen females. Ages ranged from three months to three years.

The average results from both kennels are given in the Appendix pages 25 to 26.

The tables given show the main estimations carried out in this work. Other values such as those for the differential white cell counts and specific gravities showed no correlation to the sex of the animal and were therefore not included in the tables.

The results obtained from both Kennel I and Kennel C showed that the mean red cell counts were higher in females than in males up to the age of one year. However, the highest results from male dogs always exceeded the mean values obtained from female dogs and no significance can be placed on the apparently higher red cell counts of females.

Similarly /

Similarly the values for packed cell volumes and haemoglobin were higher in females aged less than one year but again not significantly higher.

The results for dogs aged one year or more from both sets of Kennels indicated that red cell counts, packed cell volumes and haemoglobin tend to be higher in males than in females, but the t test showed that these differences were insignificant.

No correlation between the sedimentation rate and sex was observed, although the sedimentation rates for males aged less than six months appeared higher than those for females in Kennel I. This result was not confirmed by the results from Kennel C.

The total white cell count fluctuated considerably but no definite correlation with sex was found.

Platelet counts were higher in female dogs of all ages from Kennel I than in the males. Results from Kennel C showed that adult females had higher counts than adult males. In this latter establishment the males under one year of age had higher counts than females. As before, the t test indicated that the differences were not statistically significant. Anderson and Gee (1958) obtained higher values for platelets from adult females than adult males.

Care was taken not to include females who had just had litters in the results for this section. As will be seen later, in the section dealing with pregnancy and oestrus, marked changes in /

in platelet counts occur, and these changes persist for some time after whelping. In kennels having a large number of breeding females it may be found that the platelet counts are in fact significantly higher in females than in males.

The results obtained support the work of other authors, Leichsenring et al (1932), Adler (1940), Morris et al (1940), Mulligan (1941A), Van Loon and Clark (1943), Afonsky (1955) and Irfan (1958), who have stated that there is no variation in the blood picture due to sex differences. Authors such as Wintrobe et al (1936) and Landsberg (1942), who attribute differences in the blood picture to sex, have given results similar to those presented here. Their results do not appear to show any statistically significant differences.

CONCLUSIONS

The conclusions from this work show that no statistically significant changes occur in the blood of dogs due to sex differences. However, there is a tendency for the platelet counts of adult females to be higher than those of adult males, especially if they have been used for breeding. There is a tendency also for the red cell counts, packed cell volumes and haemoglobin to be higher in adult males than in adult females, but lower in males of under one year of age than in females of a similar age. These differences are very small.

For the purposes of this work changes associated with pregnancy and oestrus are not considered as changes due to sex.

VARIATIONS DUE TO AGE

Considerable work has been done on this aspect of the blood picture in dogs, and changes have been shown to occur. This work was done to confirm the results of previous authors, and also to find out what changes occur in individual dogs as they age - somethigg which has not appeared in the literature before. In addition work was also done to see if similar changes associated with ageing occur in dogs from one source when compared with dogs from another source. This aspect has never been investigated before.

PRESENT WORK

As sex has already been shown to cause no statistically significant variations in the blood picture, dogs of both sexes were compared. The problem was tackled in two different ways in order to try and remove variations caused by factors other than age.

Firstly, a litter of six greyhound puppies was sampled between the ages of six weeks and over one year. Code numbers C.8 to C.13 inclusive.

Two other litters of dogs, code numbers S.16 to S.18, and S.19 to S.22 were sampled at various intervals, and an individual dog P.77 was also sampled.

Secondly, whole kennels of dogs, sub-divided into age groups, were compared with dogs of the same age from other kennels. For this purpose, dogs from Kennels I, Pet dogs and working dogs were used. These dogs were used to establish any changes seen in adult life.

RESULTS /

RESULTS

VARIATIONS DUE TO AGE IN GREYHOUND PUPPIES (C.8 to C.13)

These dogs, four males and two females were housed together and fed in an identical manner during the period of observation. The only variations in management which occurred were when dogs C.11 and C.12 were put into training at the age of one year two months. The other dogs were not trained during the period of observation. The individual results for these dogs are not given but average values together with ranges of variation and standard deviations are given in the text.

Variations in the blood picture occurred in these six dogs as they aged.

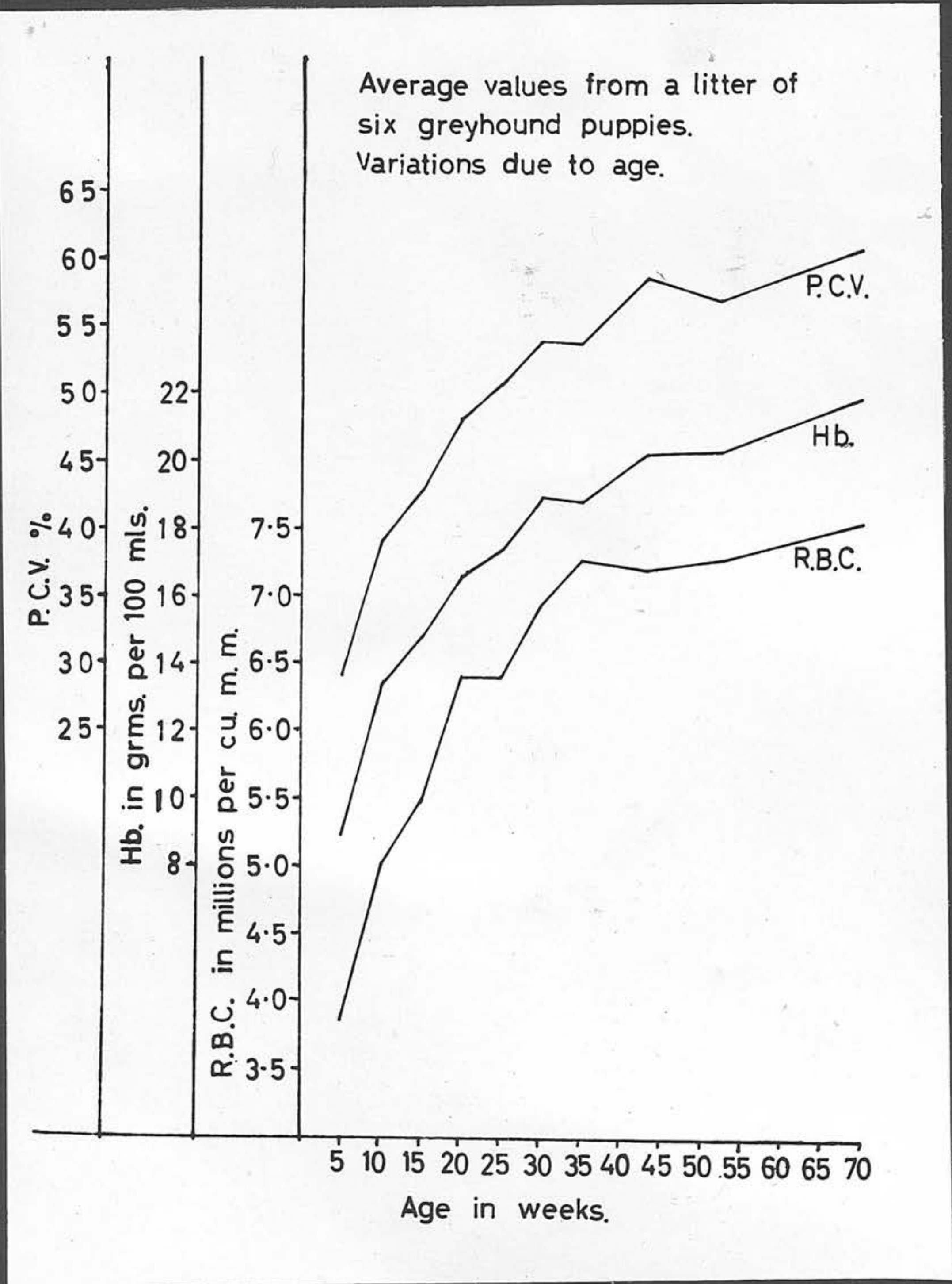
Variations in red cell counts, haemoglobin, packed cell volumes and specific gravity.

These four values tended to follow the same pattern and will be discussed together.

At the age of five and a half to six and a half weeks, when sampling commenced, the average values obtained were as follows:-

	R.B.C. millions per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	Specific Gravity
Average	3.840	29	8.9	1.0403
Standard Deviation	± 0.367	± 2.72	± 0.80	-
Range of Variation	3.265 - 4.250	24 - 32	7.8 - 10.2	1.038 - 1.0415

These values rose steadily in all six dogs until at the age of twenty-nine to thirty and a half weeks the following average results were obtained:



Average values from a litter of six greyhound puppies. Variations due to age.
P.C.V.
Hb.
R.B.C.
P.C.V. %
65
60
55
50
45
40
35
30
25
Hb. in grms. per 100 mls.
22
20
18
16
14
12
10
8
R.B.C. in millions per cu. m.m.
7·5
7·0
6·5
6·0
5·5
5·0
4·5
4·0
3·5
5 10 15 20 25 30 35 40 45 50 55 60 65 70
Age in weeks.

FIG. 1

	R.B.C. millions per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	Specific Gravity
Average	6.917	53.6	18.9	1.0637
Standard Deviation	± 0.407	± 2.35	± 0.66	-
Range of Variation	6.465 - 7.370	51 - 57	18.1 - 19.6	1.0615-1.0665

From the age of thirty weeks to the age of one year the values again rose, but not so sharply as before. At fifty-two to fifty-three weeks of age the following values were obtained:-

	R.B.C. millions per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	Specific Gravity
Average	7.258	56.6	20.2	1.0669
Standard Deviation	± 0.116	± 1.24	± 0.32	-
Range of Variation	7.150-7.370	54.5-58.0	19.7 - 20.7	1.0665-1.0680

These results clearly show that there is a statistically significantincrease in all the values under discussion between the ages of six weeks and six months. After the age of six months a further increase occurs up to one year. This increase is not statistically significant but is clinically significant. After the age of one year the values fluctuate but no increase was observed and they appear to have reached a steady adult value.

The increases in all the values under discussion is shown in the graph. Fig. 1.

Variations in total and differential white cell counts, and reticulocyte counts

These values will be dealt with in the same way as the red cell values. At the age of five and a half to six and a half weeks the absolute results were the following:

	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.	Retic. %
Average	12217	6312	4630	272	833	169	5.2
Standard Deviation	± 1142	± 865	± 734	± 82	±476	± 60	± 2.65
Range of Variation	10395-13585	5230-7402	3378-5603	151-348	348-1325	87-272	2.8-9.8

At the ages of twenty-nine to thirty and a half weeks the values had altered as follows. For Reticulocytes the values are for nineteen weeks of age.

	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.	Retic. %
Average	8254	4218	3076	294	571	93	0.18
Standard Deviation	± 799	± 501	± 797	-	-	-	± 0.044
Range of Variation	7750 - 9825	3748-5025	2090-4673	197-418	335-737	Nil-202	1 - 2

The differences in total white counts between the two age groups are statistically significant and show that the white cell count is higher in pups of six weeks old than in dogs of six months of age. It must, however, be stated that there is no

significant /

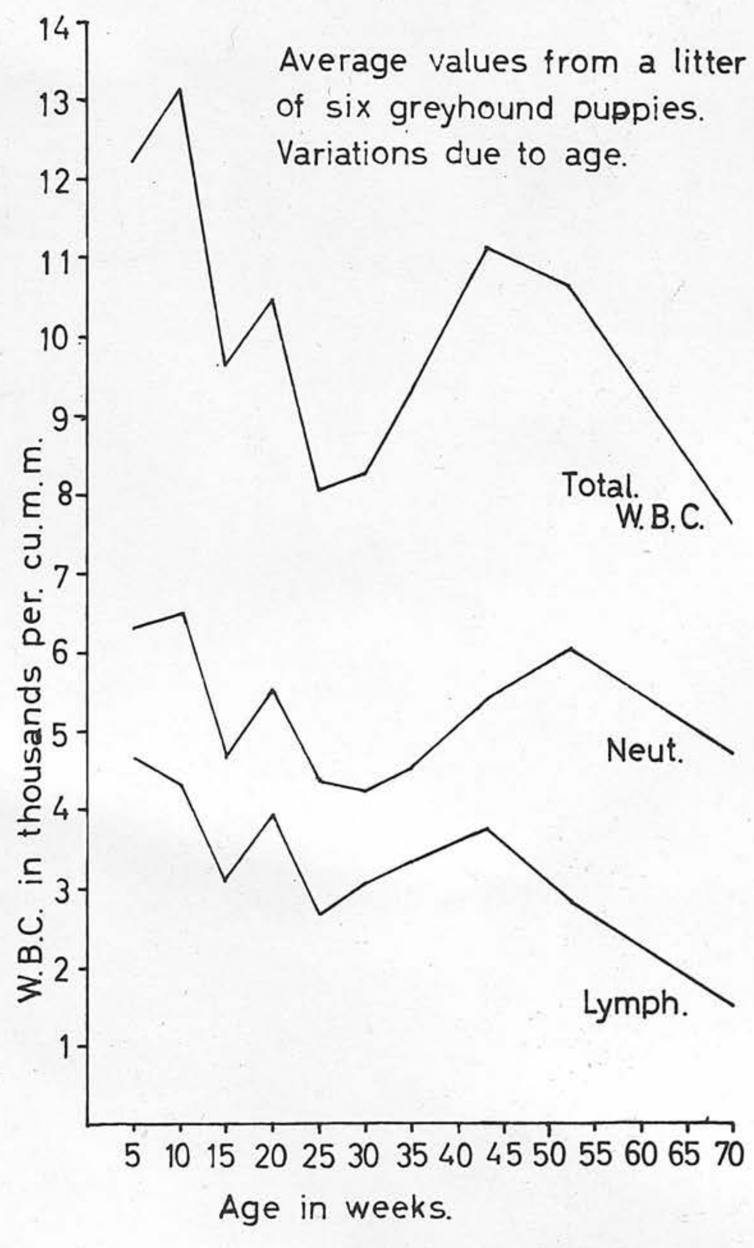

Average values from a litter
of six greyhound puppies.
Variations due to age.
14
13
12
11
10
9
8
7
6
5
4
3
2
1
W.B.C. in thousands per. cu.m.m.
Total.
W.B.C.
Neut.
Lymph.
5 10 15 20 25 30 35 40 45 50 55 60 65 70
Age in weeks.

FIG. 2

significant differences in total white counts between dogs of six weeks and three months of age, or between dogs of three and six months of age. No differences in white cell totals between dogs aged six months and those of one year was observed.

The graphs (Fig. 2) show the fluctuation in total white cell counts which occurred, but until the results from other kennels, in relation to white cell counts, have been examined, no definite conclusions can be drawn from this isolated result.

The differential white cell counts show that both the number of neutrophils and the number of lymphocytes fall as the animal ages, but these changes are not significant from a statistical viewpoint. The other cells show alterations but again these are not significant.

The reticulocyte counts fall very significantly from the age of six weeks and approach a stable adult value between the ages of three and four months.

The results obtained from dogs of one year old are as follows:-

	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lympho. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.
Average	10625	6076	2819	354	1232	142
Standard Deviation	± 1715	± 1555	± 394	-	-	-
Range of Variation	8250 - 13200	3960 - 8280	2268 - 3294	288 - 528	480 - 2442	Nil - 240

The total white cell counts did not show any significant differences in dogs of one year old from the white cell counts of younger /

younger dogs. No significant variation was found in the differential white cell counts although the lymphocytes are generally lower in adult dogs than they are in pups of six weeks old, and the absolute lymphocyte figures did fall as the dogs got older.

No variations due to age were noticed in the mature and immature neutrophils.

The fluctuations seen in the total and differential white cell counts during the first year of life are shown in the graph (Fig. 2).

Corpuscular Values

Another variation which was seen concerned the M.C.H.C. values. In dogs of six weeks and six months of age the following values were obtained:

	6 weeks M.C.H.C. %	6 months M.C.H.C. %
Average	30.9	35.2
Standard Deviation	± 1.28	± 1.22
Range of Variation	29 - 32.5	34.2 - 36.3

These differences are not statistically significant, but in all dogs the M.C.H.C. values tended to be lower in the younger age groups. No other corpuscular values showed any change with age.

Variations /

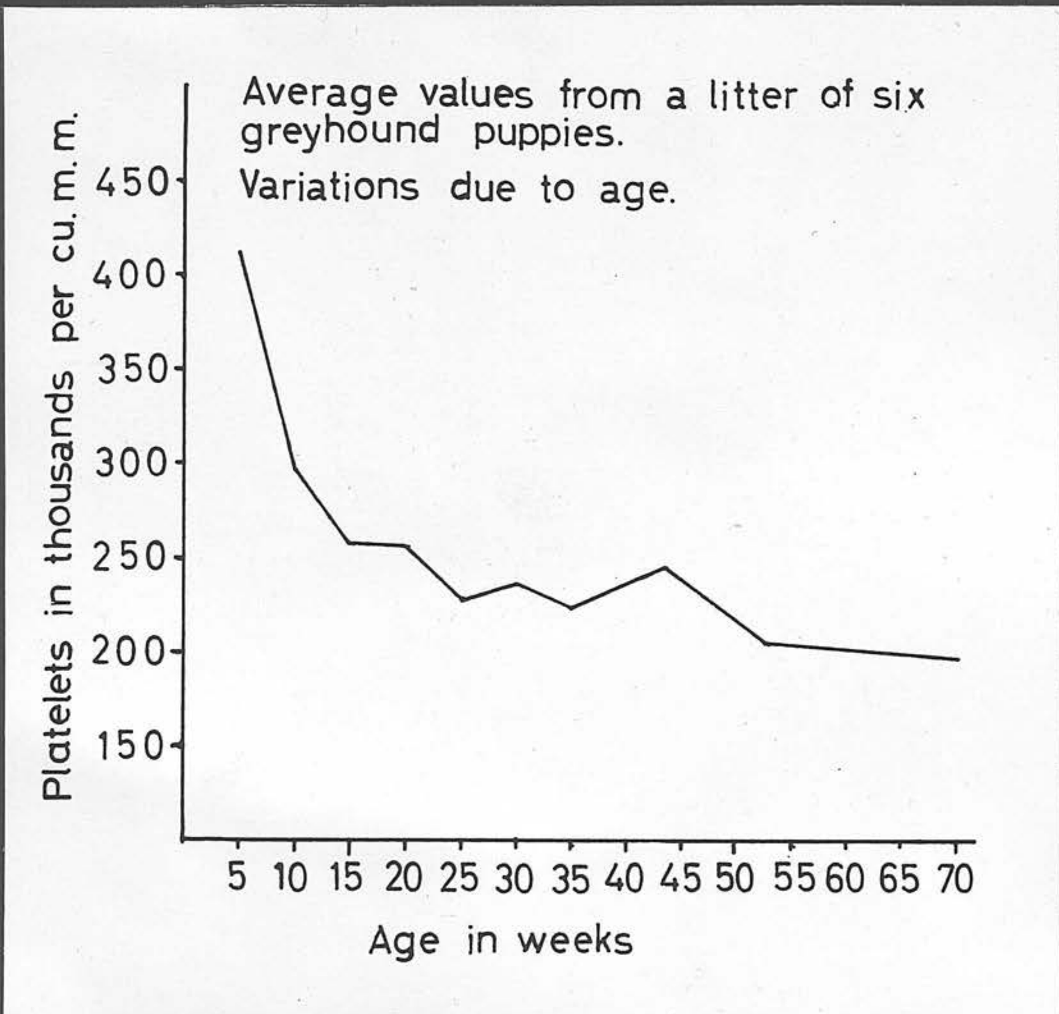

Average values from a litter of six greyhound puppies.
Variations due to age.
Platelets in thousands per cu. m.m.
450
400
350
300
250
200
150
5 10 15 20 25 30 35 40 45 50 55 60 65 70
Age in weeks

FIG. 3

Variations in Platelet Counts

The platelet counts in all six dogs showed a decrease from the age of six weeks to one year. The results obtained are as follows:-

	5-6 weeks of age Platelets thousands per cu.m.m.	1 year of age (five dogs) Platelets thousands per cu.m.m.
Average	411	205
Standard Deviation	± 85	± 17.6
Range of Variation	314 - 552	177 - 222

These results show that the decrease is a significant one as the t test gives a result of greater than 1. It must be noted that the decrease in platelets was very rapid between the ages of six weeks and fifteen to twenty weeks. Hereafter the fall was slight. No significant variation between the platelet counts of dogs aged three or four months and those aged one year could be shown. The graph shows this clearly. (Fig. 3).

In order to confirm the results obtained from the litter of greyhounds, seven other dogs from another Kennel were sampled. These dogs were all beagles and came from two separate litters. The first litter consisted of three dogs, code numbers S.16 - S.18 and the second of four dogs, code numbers S.19 - S.22. These dogs were only sampled up to the age of thirty weeks.

KENNEL S.

Dogs S.16 - S.18

Variations in red cell counts, packed cell volumes and haemoglobins

The results are again divided into those obtained at six weeks and thirty weeks of age. These are as follows:-

Six weeks of age

	R.B.C. millions per cu.m.m.	P.C.V. %	Hb. grms/100 mls.
Average	4.846	35.3	12.6
Standard Deviation	± 0.105	± 1.51	± 1.05
Range of Variation	4.740 - 4.950	34 - 37	11.9 - 13.8

Thirty weeks of age

	R.B.C. millions per cu.m.m.	P.C.V. %	Hb. grms/100 mls.
Average	6.915	51.1	19.1
Standard Deviation	± 0.567	± 1.53	± 0.52
Range of Variation	6.280 - 7.370	49.5 - 52.5	18.9 - 19.7

All these results agree with those from Kennel C in that there is a significant rise in all values from the age of six to thirty weeks. In these three dogs the rise was not as uniform

as /

as in the greyhounds. Between the twelve to twenty week period fluctuations occurred, and in two dogs the values fell between the twelfth and fifteenth week. In only one of the three dogs was the M.C.H.C. lower initially than at thirty weeks of age and no significance can be placed on this.

The specific gravity values increased with age.

Variations in total and differential white cell counts, reticulocytes and platelet counts

The following results were obtained.

Six weeks of age

	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymph. per cu.m.m.	Retic. %	Platelets thousands per cu.m.m.
Average	15473	7907	6475	2.2	443
Standard Deviation	± 3558	± 1825	± 1583	± 0.26	± 58.3
Range of Variation	17969 - 11400	9525 - 5928	7460-4645	2.5 - 2.0	506 - 391

Thirty weeks of age

	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymph. per cu.m.m.	Retic. %	Platelets thousands per cu.m.m.
Average	10583	5395	4304	0.4	308
Standard Deviation	± 1268	± 882	± 352	± 0.10	± 43
Range of Variation	11425 - 9125	6227-4471	4620-3924	0.5 - 0.3	333 - 258

The falls in total white cell counts, and in neutrophils and lymphocytes between six weeks to thirty weeks of age, are not significant. If, however, the results for dogs S.16 and S.17 are taken alone the falls in total white counts and lymphocytes are significant. It was only dog S.18 which showed virtually no change in any of these values. The results for this dog are the lowest values for white cells, neutrophils and lymphocytes shown in the ranges of variation. This result tends to confirm the results obtained from the greyhounds where the falls were more marked in some dogs than in others.

The /

The fall in reticulocytes was significant and adult values were reached at about thirty weeks of age.

The decrease seen in platelet counts in this group of dogs was not significant, unlike the greyhound results. This was due to the wide variation seen in platelet counts for dogs aged six weeks.

RESULTS FROMLITTER MATES S.19 - 22

The results for dogs S.19 - 22 inclusive appear in the Appendix page 27. These dogs were bled during the ages of fourteen and twenty-six weeks of age only.

The values obtained indicate that the red cell counts, haemoglobin and packed cell volumes rose but in an erratic manner, and in dog S.22 the values for red cell counts actually decreased. Similarly the total white cell counts and platelet counts tended to fall but not significantly so, and in dogs S.19 and S.22 the white cell count rose slightly, and in S. 22 the platelet counts did not alter.

Of the seven dogs described from Kennel S, six showed increases in red cell counts, haemoglobin, and packed cell volumes as they got older, while one fell slightly. Six showed falls in platelet counts; four had falling total white counts, but only three showed decreasing neutrophil and lymphocyte counts as their ages increased.

RESULTS FROM DOG P.77

Dog P.77, the author's own dog, and one which has always been clinically /

clinically healthy, and is still healthy at the age of almost three years, showed the most erratic changes in her blood picture between the ages of nineteen and fifty-three weeks. No significant variations in any of the values under discussion occurred during this period. The complete results for this dog appear in the Appendix page 28.

The results obtained from small groups of litter mates and an individual dog have been in some aspects inconclusive. Therefore the effect of age on the blood picture of whole groups of dogs will now be discussed and this will include the results obtained from dogs of more than one year of age.

AGE VARIATIONS IN DOGS FROM KENNEL I.

The dogs are classed into four groups for the purposes of this work. The four groups are three to four months, five to six months, one to two years and two to four years of age. Both sexes are included.

Variations in red cell counts, packed cell volumes and haemoglobin values

The average results are given in the Appendix page 29. These results confirm clearly the rise in red cell counts, packed cell volumes and haemoglobin which occurs up to one year of age.

The difference between three and six months was not significant with regard to red cell counts, but was significant for packed cell volumes and haemoglobin. There was a significant variation between the values from three month old dogs and those from dogs aged one year or over.

No clearly significant variations occurred between dogs of six months of age and those of one year, nor between those of one year and over one year, but there was a tendency for the values to increase.

Speeific gravity values increased significantly as the dogs got older and levelled out to a steady value between six months and one year of age.

Reticulocytes showed little variation - the significant decrease occurring before three months of age.

Variations /

Variations in total and differential white cell counts and platelet counts

The average results are shown in the table Appendix page 30.

The standard deviation was not worked out as it was quite obvious that no significant changes occurred in any of these values. The decrease in white cells, neutrophils and lymphocytes seen in the majority of the dogs already discussed was only just appreciable in the dogs from Kennel I, and the platelet counts showed no change. The failure to obtain similar results in dogs from this kennel is probably due to the age changes being masked by variations due to environmental factors. This aspect will be discussed in full in the section dealing with environmental changes.

Variations in Sedimentation Rates, Reticulocyte counts and Corpuscular values.

The following results were obtained.

Age	Sedimentation Rate m.m. per hour	Reticulocytes %	M.C.H.C. %	
3-4 months	7	1.5	31.8	Average
	3 - 10	0.5 - 2.2	30.5 - 33.7	Range of Variation
5-6 months	2.6	1.4	34.3	Average
	Nil - 10	0.3 - 1.8	33 - 38	Range of Variation
1-2 years	Nil	0.3	34.9	Average
	Nil	0.1 - 0.5	33.5 - 36.8	Range of Variation

These /

These results show the following significant changes. The sedimentation rates were higher in the three to six months group than in adults. Reticulocytes fell to adult values at a little over six months. The M.C.H.C. values tended to be lowest in the three to four months group, but not significantly so.

VARIATIONS DUE TO AGE IN PET DOGS AND WORKING DOGS.

The pet dogs came from varied environments but were all pets living in the house. The dogs are grouped, irrespective of environmental conditions, according to age. Greyhounds are not included in this group as they tend to have different blood pictures from other dogs. Dogs under one year of age are also excluded, as this group was used to establish any changes occurring in old age. Four puppies aged one day old are included.

The working dogs also came from varied environments, but all were labradors, working as gun-dogs and living in the country.

The results for these two groups of dogs willbe dealt with together in the following age groups: one to three years, four to nine years, and lastly ten years of age and over.

RESULTS

Day old puppies

These dogs, P.8 - 11 inclusive, all came from the same litter, and the average values for these are given in full in the Appendix page 31.

Adult dogs

The average values obtained from pet dogs and working dogs are given on pages 32-34 in the Appendix.

No significant variations were seen in the blood picture of adult /

adult dogs as they aged. There was a tendency, however, for the red cell counts, packed cell volumes and haemoglobin to decrease especially after the age of eight to nine years. Lymphocytes tended to fall the older the dogs got, and also the sedimentation rates were often increased in dogs of ten years of age and over.

DISCUSSION OF RESULTS

It has been shown in the results that variations associated with age occur inthe dog's blood picture, particularly in the first year of life. In the present study, the red cell counts, haemoglobin values and packed cell volumes were low at birth, and these results agree with those of Burnett and Traum (1905), Wells and Sutton (1915), Ederstrom and De Boer (1946), El Hindaway (1948) and Anderson and Gee (1958). Each author gives slightly different figures due to the different techniques employed. Reticulocyte counts were high and normoblasts present at birth, a fact noted by Burnett and Traum (1905) and El Hindaway (1948). All other values are similar to those of older dogs except for the absolute lymphocyte figures which were low, and the M.C.V. values which were much higher than for older dogs. Ederstrom and De Boer (1946) also showed that the M.C.V. values are higher in new-born puppies than in older dogs. Burnett and Traum (1905) give absolute lymphocyte figures which are in the low range (2762 - 743 per cu.m.m.) for new born puppies. The results given here confirm these findings.

Variations in red cell counts, packed cell volumes, haemoglobin and specific gravity

The results obtained from the litter of greyhounds show that the red cell counts, packed cell volumes, haemoglobin values and specific gravities all increase steadily until about thirty weeks of age. After this age the increases continued but were more erratic. The average increase in red cell counts between the ages of /

of six weeks and one year was almost one hundred per cent, for packed cell volumes just over one hundred per cent and for haemoglobin one hundred and twenty per cent. These results show the same definite trend as the results of Mezincescu (1902), Burnett and Traum (1905), Wells and Sutton (1915), Landsberg (1939 and 1942), Morris et al (1940), Mulligan (1941B), Ederstrom and De Boer (1946), Anderson and Gee (1958) and Irfan (1958).

El Hindaway (1948) was unable to show such large increases as are seen in the present results, probably because the dogs used were strays.

The results do not agree with those of Adler (1940) or Van Loon and Clark (1943) who found that no variations occurred as the dogs aged. None of the workers mentioned gave results for specific gravities.

Reticulocytes decreased as the dogs aged and reached adult values at about fifteen weeks of age.

The results obtained from the two litters of beagles from Kennel S and the beagles from Kennel I substantiate the results obtained from the greyhounds with regard to red cell counts, packed cell volumes, haemoglobin values and specific gravities.

All these values increased up to one year of age, the most rapid/ increases occurring between six weeks and six months of age.

The results from these sources and especially from dogs S.18, S.19 and S.22 showed increases which were not as steadily progressive /

progressive as those from the greyhounds. Erratic variations were seen especially between the ages of three and five months. These erratic variations are considered to be due to the effects of environmental factors and individual variations and not to age. These will be discussed in the section dealing with environmental changes.

The results from dog P.77 appear to be at variance with other results due to the individual variation of ths particular animal. Individual variations were seen in the greyhounds when the results of one dog were compared with another.

The results from Kennel I showed a steady increase in all values up to the age of one year. The environmental factors present in these kennels do not seem to have had any effect on the red cell counts, packed cell volumes or haemoglobin values. These factors will be discussed later. (Effects of environment).

The results in adult dogs from Kennel I, Pet dogs and Working Labradors, showed similar findings. Adult values are reached at approximately one year of age and remain fairly constant after this, although there is a tendency for the values to fall in dogs over eight or nine years of age. Considerable individual variations are seen and the ranges of variation for any age group are wide.

Variations in total and differential white cell counts and platelet counts

The decrease in total white cell counts between the ages of six weeks and thirty weeks shown by the litter of greyhounds, is not /

not thought to be of as much significance as it appears at first sight. The white cell counts fluctuated considerably between the ages of ten and forty-five weeks of age and the fact that the differences were significant is only a matter of chance. They would have been insignificant if the figures for thirty-five weeks of age had been chosen instead of those for thirty weeks of age. There was an overall decrease in the total number of white cells, neutrophils, and lmyphocytes as the dogs got older, but this decrease was erratic and not statistically significant. The findings of Burnett and Traum (1905), Wells and Sutton (1915) agree with the above. Morris et al (1940) found that the lymphocytes were higher in puppies than in adults as did Irfan (1958), while Adler (1940) and Irfan (1958) considered that total white counts tended to be higher in young dogs than in adults.

The results of Mezincescu (1902) and Anderson and Gee (1958) showed little variation in total white or differential counts as the dog aged, while El Hindaway (1948) gave higher values for adults than puppies. Anderson and Gee's results show signs of environmental influence, which mask the changes due to age to some extent.

The fall in platelet counts from the age of five to fifteen weeks was not seen by El Hindaway (1948).

The results for dogs of one year and under from Kennel S, agree /

agree with those from the greyhounds and show a tendency, which is not statistically significant, for total white cell counts, lymphocytes, and neutrophils to fall as the dogs got older. In five out of the seven dogs, the platelet counts fell as the dogs got older, but the fall took longer to occur than in the greyhounds.

Again dog P.77 was a law unto herself and showed none of these changes.

The results from Kennel I do not show the same changes as other dogs. There was a slight fall in the total white cell counts but no variation in neutrophils or lymphocytes. This is due to environmental factors masking any changes associated with age. The figures for total white cell counts and neutrophils were considerably in excess of the results for any of the other dogs. They are similar to the results of Anderson and Gee (1958).

The platelet counts from Kennel I showed no alteration. These results do not agree with those of Anderson and Gee (1958) who found an increase in platelets as the dogs aged.

In the adult Pet dogs studied the figures showed marked individual variations and the only alteration which was apparent was that the lymphocytes tended to fall, being lowest in dogs of ten years of age or more.

Variations in other values with age

Sedimentation rates were slightly higher in the greyhounds aged two to three months than in older greyhounds. In dogs from Kennel /

Kennel S they were highest up to the age of four months. Neither of these results appeared significant. Dogs from Kennel I, however, showed significantly raised sedimentation rates up to the age of five months. The reason for this is not known but it was noticed that the sedimentation rates increased when the dog's permanent teeth were erupting. This fact was noted by Anderson and Gee (1958).

Sedimentation rates remained consistently low until the age of ten years after which there was a tendency for the rates to increase, probably due to senile changes.

CONCLUSIONS

The results of this work show that in dogs not subjected to adverse environmental factors such as sub-clinical disease, the red cell counts, packed cell volumes, haemoglobin values and specific gravities all increase significantly and steadily from birth to the age of about one year. The most rapid increases take place from birth to six months of age approximately.

There is a tendency for the total white cell counts, neutrophils and lymphocytes to fall from the age of two months to one year.

Platelet counts are often higher in puppies of two to three months of age than in dogs of six months of age. Sedimentation rates tend to be higher in puppies under four or five months of age than in young adults. Reticulocyte counts are significantly higher at birth and fall to low adult values at about four months of age.

No significant variations occur in normal adult dogs but lymphocytes tend to decrease with age, and sedimentation rates to increase after the age of ten years.

All these changes can be masked or altered by the influence of environmental factors. Some dogs do not follow the general trend owing to individual idiosyncrasies which apply to that dog alone. The actual values obtained from dogs in a particular age group vary from dog to dog, even ones from the same litter, and from kennel to kennel.

VARIATIONS DUE TO ENVIRONMENT

In the previous section dealing with age variations mention has been made of the effect of environmental factors. Only one piece of recorded work on this subject has been traced, although it is generally known that such factors do affect the blood picture. Afonsky (1955) gave average results for dogs obtained from three separate kennels. These results showed variations but no attempt was made to assess the differences. All the kennels housed experimental dogs.

PRESENT WORK

As age has already been shown to affect the blood picture the dogs used are grouped in age groups. Where possible, dogs of one breed and age, living in one kennel, were compared with dogs of the same breed and age from other kennels. As well as this, dogs of one breed and age living in kennels were compared with dogs of the same breed and age living in private houses, and in the same way dogs living in the town have been compared with dogs living in the country A kennel of dogs of mixed breed has also been compared with pets of mixed breeds. Both sexes are used.

For this section, dogs have been obtained from the following sources:- Kennel I, Kennel C, Kennel H, Pet Dogs and Kennel S.

RESULTS

Kennel I. (Beagles)

This kennel is the one which shows the mostmarked variations due /

due to environmental factors. The kennel housed very large numbers of dogs. Infectious diseases were only kept at bay by constant vaccination and the majority of dogs, especially younger ones, suffered from ascarid infestations, despite routine deworming, and also demodectic mange. The average results obtained are given in the Appendix page 35.

KENNEL S (Beagles)

This kennel is smaller than Kennel I. Routine vaccination kept infectious diseases at bay, and ascarid infestation was light. Demodectic mange appeared occasionally but was treated before the lesions became larger. The average results from this Kennel are given in the Appendix page 36.

Comparison of the results from these two kennels show that variations occurred, due to the effect of environmental factors.

Packed cell volumes, haemoglobin and sedimentation rates

The results for packed cell volumes and haemoglobin at three to four months of age were higher in beagles from Kennel S than those from Kennel I. The difference was not statistically significant. The results remain higher until the age of two years when the results for both kennels were almost identical, and they remained like this from two years upwards. This difference is due to the fact that thebeagles in Kennel S got more individual attention and more food than those in Kennel I with the result that they put on weight faster and had higher haemoglobins and more red cells. The food given in Kennel I was excellent but young dogs were /

were fed together in groups of ten to twenty dogs with the result that the smaller dogs did not get their fair share. In consequence the average weights, haemoglobins, red cell counts and packed cell volumes tended to be lower than those for the beagles in Kennel S.

The average weights for dogs aged three to four months and four to five months from the two kennels are as follows:

Age	Kennel I.	Kennel S.
3 - 4 months	14.1 lbs. (6 dogs)	15 lbs. (6 dogs)
4 - 5 months	14.6 lbs. (5 dogs)	16.3 lbs. (6 dogs)

After the age of six months dogs retained for breeding got much more individual attention in Kennel I owing to the smaller numbers involved. As a result their weights and red cell counts rose more rapidly and caught up with those in Kennel S by one year of age.

The sedimentation rates tended to be higher in young puppies in Kennel I than those in Kennel S. The cause is not known with certainty.

Total White Cell and Differential White Cell and Platelet Counts

In both Kennels the total white cell counts were high at the age of three to four months, being highest in Kennel I. These values fell erratically in Kennel S as the dogs got older, falling from 15275 ± 3242 per cu.m.m. at three to four months of age to 7615 ± 1740 per cu.m.m. in the two to four years age group. This fall is not statistically significant owing to the wide ranges of variation, but is clinically significant.

In /

In Kennel I the total white cell counts varied from age group to age group and tended to fall but not to the same extent as Kennel S. They fell from 19154 per cu.m.m. at three to four months of age to 14424 per cu.m.m. at two to five years of age. This decrease is not statistically significant.

The reason for the total white cell counts remaining high in Kennel I is the constant stimulus to white cell production caused by the presence of sub-clinical infections and parasitic diseases, andalso possibly to minor wounds caused by fighting. This is shown clearly in the fact that dog I.15 had a total white cell count of 22,550 per cu.m.m. when first sampled. She was then transferred to Edinburgh and eventually went to Kennel S as dog S.24. Her white cell counts fell within one month of leaving Kennel I and have remained in the region of 9000 - 12000 per cu. m.m. since (when not pregnant).

In dogs from Kennel I neutrophil counts fell only very slightly and lymphocytes showed little alteration until after two years of age, when they also fell slightly. Both neutrophils and lymphocytes started to decrease slowly in dogs from Kennel S after the age of six months.

Neutrophils, lymphocytes and eosinophils were higher in dogs from Kennel I than in those from Kennel S in nearly all age groups, but not significantly so.

Platelet counts were generally higher in dogs from Kennel I than in those from Kennel S.

Some /

Some information not given in the previous tables can be mentioned here.

Blood samples were taken from four beagles aged nine months of age from Kennel S, and very high white cell counts were obtained. These were much higher than the average results for dogs from this kennel.

The dogs were Nos. S25, S.26, S.27 and S.31. All appeared to be in good bodily condition, eating well, very lively and apparently perfectly normal. The results obtained were as follows:

Dog	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymphs. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.
S.25	20850	9695	9174	1147	730	104
S.26	14550	6766	6475	1018	291	-
S.27	18200	9236	6734	1319	819	91
S.31	20125	11270	6540	906	805	603

In order to make sure that these results were correct repeat samples were taken plus samples from two adult dogs and another nine month old dog (S.28) not previously sampled, with the following results:

Dog	W.B.C. per cu.m.m.	Neuts. per cu.m.m.	Lymphs. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.
S.25	16850	7498	7582	927	421	421
S.26	17500	7962	8138	787	525	87
S.27	16875	7341	6412	1265	1603	253
S.31	15700	7300	6672	1177	392	157
S.28	11275	7611	2255	958	225	225
S.23	6350	3778	1841	381	286	63
S.3	7450	4656	1825	633	186	149

The adult dogs and the newly sampled nine month old dog showed lower counts than the four original dogs.

If the four sets of results are compared with the average values for dogs aged nine months from Kennel S it is obvious that the values are much higher.

Average values obtained.

	W.B.C. per cu.m.m.	Neut. per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.
1st Sampling	18431	9241	7230	1097	661	199
2nd Sampling	16731	7525	7201	1039	735	229

Average values for dogs aged nine months in Kennel S.

W.B.C. per cu.m.m.	Neut. per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Misc. per cu.m.m.
12200	6814	4019	749	441	176
9195 - 14550	5885 - 7806	2255 - 6475			

The total white cell counts, neutrophils, lymphocytes and monocytes were elevated, at the first sampling. There was no shift to the left. At the second sampling the total white cell counts had fallen but were still above the average values for this kennel. The neutrophils also fell, but lymphocytes and monocytes remained high. It would appear that these dogs had been subjected to some stimulus causing a rise in total white cell counts, especially lymphocytes. The most likely cause is some infectious disease to which the dogs were already partly immune by reason of vaccination. I was unable to prove this, but adult dogs sharing the same kennels showed /

showed no change in their white cell picture - the unknown stimulus obviously did not affect them as it did the younger dogs. One nine months old dog (S.28), again sharing the same kennel, also showed no alteration in its white count although its kennel mate's white count was elevated. One can only assume that this dog was also completely immune.

The blood pictures of these four dogs illustrate how apparently normal dogs can show marked changes, especially in their white counts, without showing clinical signs, and that such changes can affect one dog and leave another unaffected. This type of change is seen most readily in kennels where large numbers of dogs are housed together.

KENNEL C AND PET GREYHOUNDS

Having compared two kennels of dogs, results from another kennel will be compared with those for dogs of the same age and breed living singly or in small groups. For this greyhounds from Kennel C were compared with other greyhounds. As it was not possible to obtain "pet" greyhounds of less than six months of age, only results from dogs of six months of age or over from both groups will be given. The "pet" greyhounds lived in private houses or in small groups from various sources. Many of them were racing dogs. The average results for both these sets of greyhounds are given in the Appendix pages 37 and 38.

No differences of any significance were evident on comparison of the results from these two groups of greyhounds.

The /

The haemoglobin and packed cell volumes from dogs in Kennel C, under the age of two years, tended to be higher than the values obtained from the other group. This slight difference is due to the better feeding in Kennel C.

No variation in white cell counts occurred but it will be noted that the results are in all instances much lower than those from Kennels I and S. This will be discussed later.

KENNEL H, and TOWN AND COUNTRY PETS

The last part of this section is a comparison between mixed breeds of dogs of the same age from three different sources which are as follows.

Kennel H.

This kennel was situated in a town and the population was frequently changing as some of the dogs were only boarders. This kennel has been fully described previously. The results given in the Appendix page 39 do not include those for beagles living in these kennels.

Pet Dogs

The results from Kennel H will be compared with the results from pet dogs. The pet group is divided into two sections. The first section consists of pet dogs of mixed breeds living in the country. Many of these dogs are labradors living in houses but used as gun-dogs. As work seems to have no effect on their blood picture (see later) they are used in this section.

The second section consists of pets living in the city (Edinburgh) and these are again mixed breeds. No greyhounds are included.

Both sets of results are given in the Appendix pages 40 and 41.

No variations of any significance were noted in the haemoglobin values or packed cell volumes between the dogs in these three sets of kennels.

The variations in total and differential white cell counts between the three sets of dogs are not statistically significant but a trend was noticed. From the age of nine months or more the lowest white counts from dogs in Kennel H were in the region of 8,500 per cu.m.m. and the highest values 15,700 per cu.m.m. In the same age range the lowest white count for pet dogs living in the town was 5,425 per cu.m.m. and the highest 16,725 per cu.m.m. while the lowest for country dogs was 5,125 per cu.m.m. and the highest 13,300 per cu.m.m.

The average total white cell counts for dogs of nine months of age and over were highest in dogs from Kennel H, and lowest in pet dogs living in the country. The average total white cell counts for all dogs of nine months of age and over are as follows:

	Kennel H	Town Pets	Country Pets
Average W.B.C. per cu.m.m.	11,258	10,467	9,500

Average neutrophil counts were highest from Kennel H. but there was little difference between town and country dogs. Lymphocytes on the other hand showed little variation. Average results from all dogs over nine months are as follows:

	Kennel H.	Town Pets	Country Pets
Neutrophils per cu.m.m.	7,825	6,759	5,667
Lymphocytes per cu.m.m.	2,249	2,585	2,534

No other variations were apparent between the three sets of dogs.

DISCUSSION OF RESULTS

In the results which have just been described no statistically significant differences have been seen in the groups of dogs which were compared with one another. However, a trend has been noticed especially with regard to white cell counts. If the results from all the groups of dogs are compared a more definite picture emerges.

Comparison of haemoglobin values and packed cell volumes.

It has already been noted that these values were slightly higher in young beagles from Kennel S than those from Kennel I and this difference has been attributed to the difference in feeding methods used in the two kennels. In adults the values were slightly higher in Kennel I due to lack of water. (See later).

The results obtained from the two groups of greyhounds showed haemoglobin and packed cell volumes which were higher than those obtained from either group of beagles or from mixed breeds of pet dogs or Kennel H. The total red cell counts, although not given, are in line with the haemoglobin and packed cell volumes.

The average results for haemoglobin and packed cell volumes from dogs aged nine months or more from all sources are as follows:

Age	Value	Beagles Kennel I.	Beagles Kennel S.	Greyhounds Kennel C.	"Pet" Grey-hounds	Kennel H.	Town Pets	Country Pets
9 mths. to 1 year	Hb. gms. per 100 mls.	16.9	17.4	19.6	18.5	17.1	17.4	16.8
	P.C.V. %	48.8	48.7	57.2	51.5	48.7	48.5	47.1
1 to 5 years	Hb. gms/100 mls.	18.1	18.1	21.3	20.2	18.2	18.7	18.3
	P.C.V. %	52.1	50.1	60.0	57.8	51.0	52.0	51.5
Over 5 years	Hb. gms/100 mls.	-	-	20.6	20.9	17.0	17.3	17.2
	P.C.V. %	-	-	59.6	58.9	48.0	47.7	47.7

The higher values seen in greyhounds is not due to environmental factors as might at first be suspected, but to a breed difference. This will be shown in the section dealing with breed variations.

No significant variations between the other groups of dogs was seen, although pet dogs had slightly higher values than kennelled dogs and this again is attributable to better feeding and more individual attention.

Variations in total and differential white cell counts

The variations in white cells due to environmental differences are much more marked than the variations for packed cell volumes and related estimations.

If the average total white cell count was worked out for each age group, from each kennel, considerable differences were seen. However, when the standard deviation was deduced it was found that the white cell count ranges from one kennel overlapped those from any other kennel and thus some results were not statistically significant. These variations are, however, of considerable clinical significance.

The average results together with the standard deviation is shown in the Appendix page 42. The clinical significance of the variations is best shown in diagrammatic form.

The average total white cell count for a particular age group has been found and also the standard deviation. Twice the standard deviation was then added and subtracted from the average value. In this way the complete range of white cell counts likely to be encountered in any age group, from any one kennel, was found.

For /

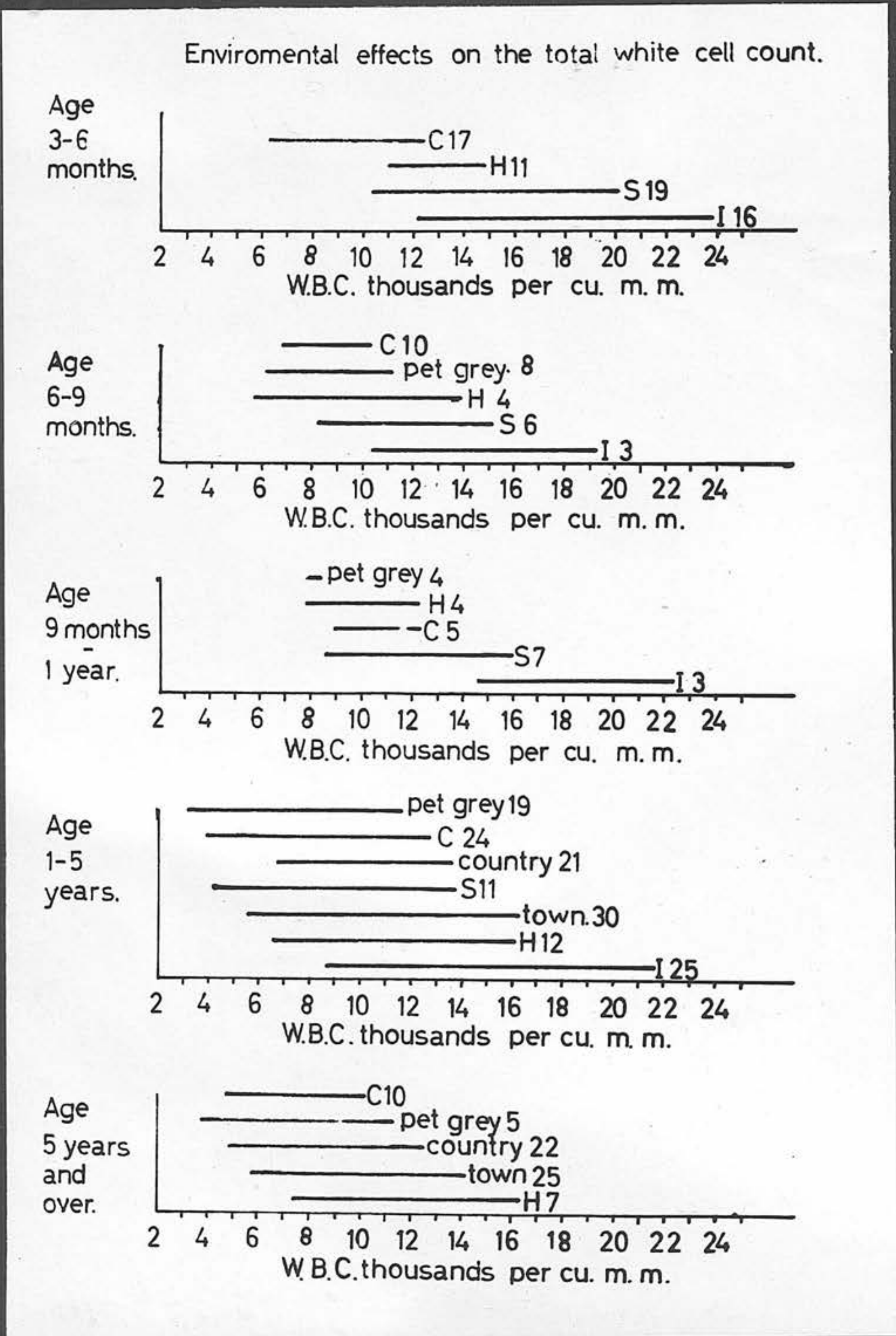

Enviromental effects on the total white cell count.
Age 3-6 months.
C 17
H 11
S 19
I 16
2 4 6 8 10 12 14 16 18 20 22 24
W.B.C. thousands per cu. m. m.
Age 6-9 months.
C 10
pet grey 8
H 4
S 6
I 3
2 4 6 8 10 12 14 16 18 20 22 24
W.B.C. thousands per cu. m. m.
Age 9 months - 1 year.
pet grey 4
H 4
C 5
S 7
I 3
2 4 6 8 10 12 14 16 18 20 22 24
W.B.C. thousands per cu. m. m.
Age 1-5 years.
pet grey 19
C 24
country 21
S 11
town. 30
H 12
I 25
2 4 6 8 10 12 14 16 18 20 22 24
W.B.C. thousands per cu. m. m.
Age 5 years and over.
C 10
pet grey 5
country 22
town 25
H 7
2 4 6 8 10 12 14 16 18 20 22 24
W.B.C. thousands per cu. m. m.

FIG. 4

For example, in Kennel I (age group one to five years), the average total white cell count was 15134 per cu.m.m. and the standard deviation ± 3224 per cu.m.m. When twice the standard deviation is added to the average, we get 21582 per cu.m.m. and when it is subtracted 8686 per cu.m.m. This gives a range of 8686 per cu.m.m. - 21582 per cu.m.m.

This calculation was done on all the age groups from all the kennels involved in this work, and the results expressed in the following diagrams. (Fig. 4).

The total white cell count is given at the base of each diagram and the range of white counts from each kennel is represented by black lines.

Some results are omitted as the number of dogs involved is too small for an accurate comparison, e.g. Town Pets aged six months to one year and country pets aged nine months to one year were there were only two dogs in each group.

The number of dogs used is given at the end of each line, and also the kennel initial. The diagrams show plainly that considerable clinical significance can be attached to these results.

This is clearly seen if the two age groups in which large numbers of dogs are involved are taken as an example, e.g. three to six months and one to five years.

In the three to six months age group a dog with a total white cell count of between 10 - 12,000 per cu.m.m. would be perfectly normal no matter from which kennel it came. With a count of 13-15,000 per cu.m.m. the dog would be normal if it came from kennels /

kennels I, S or H, but would be abnormal if it was from kennel C, and if the count were 15-20,000 per cu.m.m. the dog would be normal if from kennel I or S; abnormal from kennel H, and definitely abnormal if from kennel C. If the count was 20-24,000 per cu.m.m. the dog would probably be normal if it came from kennel I, abnormal if from kennel S, and yet grossly abnormal if from kennel C or H.

If the actual results presented are analysed for the age group three to six months, the percentage of dogs from each source which exceeds a certain white count is as follows.

Table showing the percentage of dogs from each source which exceed a given total white cell count. Three to six months age group.

Total White Count	Kennel I	Kennel S	Kennel H	Kennel C
12,000 per cu.m.m.	100%	85%	81%	5%
15,000 per cu.m.m.	94%	50%	27%	Nil
20,000 per cu.m.m.	22%	Nil	Nil	Nil
24,000 per cu.m.m.	Nil	Nil	Nil	Nil

A similar picture is seen in the one to five years age group. Dogs with white cell counts of 5-12 thousand per cu.m.m. would be normal no matter from which kennel they came. If the count was 16-22,000 per cu.m.m. the dog would be normal if from kennel I, but abnormal if from the other kennels. If the dog was a pet dog with a count of 22,000 per cu.m.m. then some gross pathological abnormal-:ity could be suspected. The percentage number of dogs from each source which exceed a given white cell count are as follows:

Table /

Table showing the percentage of dogs from each source which exceed a given total white cell count. One to five years age group.

White Cell Count	Kennel I	Kennel S	Kennel H	Kennel C	Pet Greyhounds	Town Pets	Country Pets
12,000 per cu.m.m.	88%	24%	24%	8%	5%	30%	5%
15,000 per cu.m.m.	52%	Nil	16%	Nil	Nil	10%	Nil
18,000 per cu.m.m.	20%	Nil	Nil	Nil	Nil	Nil	Nil
Over 20,000 per cu.m.m.	8%	Nil	Nil	Nil	Nil	Nil	Nil

It must be stated at this point that the low white cell counts seen consistently in greyhounds from both sources are probably conditioned both by environment and by breed. This will be shown in the section dealing with breed variations.

Having shown that the total white cell counts are influenced considerably by environment, the differential white cell counts were analysed to see which type of cells are involved.

The great majority of the dogs which showed total white cell counts in the higher ranges had high neutrophil counts, and slightly raised lymphocyte counts. Kennelled dogs had slightly higher eosinophil and monocyte counts than pet dogs.

If the three to six months age group and the one to five years age groups are taken as examples, the following average results were obtained.

	Neutrophils per cu.m.m.				Lymphocytes per cu.m.m.			
Age	Kennel I	Kennel S	Kennel H	Country Pets	Kennel I	Kennel S	Kennel H	Country Pets
3-6 months	10717	8221	7548	-	5309	5793	3827	-
1-5 years	9872	5362	7510	5939	3670	2648	2537	2776

No /

No shift to the left was seen in any of the dogs, even dogs with high total white cell and neutrophil counts from Kennel I.

CONCLUSIONS

This work has shown that definite variations occur in a dog's blood picture due to the effects of the animal's environment. These changes are important from a clinical point of view and unless environmental effects are taken into consideration, erroneous conclusions may be drawn when the white cell counts are used as an aid to the diagnosis of disease.

The following changes occur due to the effects of environmental conditions. The red cell counts, haemoglobin and packed cell volumes tend to be slightly higher in pet dogs or dogs housed in small kennels where individual attention and feeding is given, than in dogs in large kennels. These increases are due to better feeding and were found to be only slight increases and of no statistical significance and little clinical significance.

Water deprivation also causes higher results and will be discussed later. The higher results from greyhounds are due to breed differences and not to environmental effects.

The average total white cells are higher in dogs housed in large kennels than in dogs housed in small kennels, and these in turn are higher than the results from pet dogs. Pet dogs living in the country have lower average white cell counts than pets living in towns and the range of variation is less. Pet greyhounds in small kennels have low white cell counts but this is due to breed variations as well as to environment. (See later).

The /

The ranges of variation for white cell counts from pet dogs which are well looked after are smaller than for dogs housed in small kennels, and these in turn are smaller than the ranges of variation seen from dogs housed in large kennels. In consequence although the white cell counts from each source overlap there is a marked difference in the extremes of the range from each source.

In normal dogs withhigh white cell counts all the types of cells are raised but the neutrophils are elevated more than the others. No shift to the left was seen in any of the dogs investigated.

Periodic increases in white cell counts can occur in kennelled dogs due to stimuli which are not readily apparent. Not all the dogs in the kennels are affected at the same time and the dogs with high white cell counts appear clinically normal.

No significant variations due to environment were seen in any of the other estimations done on the blood, apart from the sedimentation rates in puppies from large kennels, which were higher than those in puppies from other sources. The platelet counts tended to be higher in dogs housed in large kennels. This in part at least may be due to the presence of wounds caused by fighting, and also to the fact that many of the bitches sampled were used for breeding. (See under section dealing with Pregnancy).

VARIATIONS DUE TO BREED

In the section dealing with environmental changes, mention was made of possible breed variations especially in greyhounds and beagles. In this section it is hoped to make the previous statements clear.

REVIEW OF THE LITERATURE

The majority of workers have stated that there are no variations in the dog's blood picture which can be attributed to breed differences. These workers include Scarborough (1931), Morris et al (1940), Adler (1940) and Van Loon and Clark (1943). Irfan (1958) stated that greyhounds and possibly other hounds have higher red cell counts, haemoglobins and packed cell volumes than other dogs and that beagles have higher white cell counts than other breeds.

PRESENT WORK

It was impossible to deal with all the breeds of dogs so this work has been concentrated on three breeds. These are greyhounds, beagles and labradors. An indication as to the probable blood picture of other breeds has been worked out but the number of animals involved was small.

Age and environment have already been shown to cause variations in the blood picture. All the dogs were therefore classified in age groups, and a particular breed from one kennel was compared with the same breed from one or more kennels. In this way variations due to environment could be assessed.

A /

A comparison of beagles from Kennel I, Kennel S and Kennel H was made. A comparison of pet greyhounds and greyhounds from Kennel C was made, and lastly pet labradors were compared with working labradors.

GREYHOUNDS

The complete average results for greyhounds from Kennel C and from pet greyhounds has already been given in the section dealing with environment and will not be repeated here. The average values appertaining to this section are in the Appendix page 43. From these results it is clearly seen that the red cell counts, haemoglobins, packed cell volumes and specific gravities are high in greyhounds from both sources. The range of variation was very small, even in pet greyhounds which came from many different sources, and could therefore be expected to have a wider range of variation than dogs from a single source.

All these values are consistently high for all age groups and similar results are obtained from both sources. The results from Kennel C. are slightly higher than those from the "pets" owing to the superior feeding and better management. Even in the "pet" greyhound group where many of the dogs were badly housed and fed only moderately well, the red cell counts are still high. The lowest values obtained from any adult greyhound (over 1 year) are:-

Red Cells	6,490,000 per cu.m.m.
P.C.V.	50%
Hb.	18.2 gms/100 mls.

These high results were seen in both young dogs which had never

raced /

raced, and also in old dogs which had not been in training or seen a race-track for several years. It can therefore be said that the high results are due to breed and not to racing. Low results would only be obtained from "normal" greyhounds which were very inadequately fed or housed.

The total white cell counts of greyhounds were found to be consistently low. This is understandable in dogs from Kennel C and in many of the "pet" dogs also. Some "pet" dogs, however, were housed in tin sheds on waste land. In the case of dogs P.46-53 they were well fed, but housed on waste ground in sheds. They were surrounded by other sheds and pens containing greyhounds, the great majority of which were unvaccinated, and infested with sarcoptic mange and ascarids. In such surroundings one would have expected the total white cell counts to be raised. In fact the counts ranged from 6,250-11,150 per cu.m.m.

Dogs P.106-108 belonged to an unemployed miner who could not afford to feed the dogs properly. In addition all three dogs had slight sarcoptic mange lesions. The white cell counts of these dogs was 5,000-9,500 per cu.m.m.

The highest white cell count obtained from a greyhound was 13,200 per cu.m.m. and this dog had just returned home after training.

These results indicate that greyhounds tend to have low white cell counts. These results are supported by the results of

Irfan /

Irfan (1958) who gave the average white cell counts for five breeds of adult dogs and the lowest was the greyhound with a count of 10,112 per cu.m.m.

BEAGLES

The average results for Kennel I, Kennel S and Kennel H are in the Appendix pages 44 and 45. These results showed that red cell counts, haemoglobins and packed cell volumes were similar in adult dogs from the three kennels, although the results from Kennel I tended to be higher owing to water deprivation (see section dealing with water deprivation). The average results were lower than those obtained from either group of greyhounds.

The average haemoglobin values from three sets of adult beagles was lower than the lowest value obtained from any of the greyhounds. Some of the red cell counts and packed cell volumes were higher and some lower than the greyhounds lowest value. The thirteen beagles from Kennel H have not been mentioned before but are given with the results in the following table. Their average results are given in the Appendix page 45.

Average values from Beagles compared with the lowest values from any greyhound.

	Average results from Beagles aged 1-5 years			Lowest values from any greyhound aged 1-5 years
	Kennel I.	Kennel S.	Kennel H.	
R.B.C. millions per cu.m.m.	6.981	6.424	6.589	6.490
P.C.V. %	52.1	50.1	50.0	50.0
Hb. gms/100 mls.	18.1	18.1	17.5	18.2

These results show that the average beagle aged 1-5 years has a red cell count of approximately 6.5 millions per cu.m.m. and packed cell volume of 50% and a haemoglobin of 18.0 grms/100 mls. This is much lower than a greyhound which averages 7.4 millions red cells per cu.m.m. and packed cell volume of 58% and haemoglobin of 21 grms/100 mls. Standard blood pictures for each breed will be given in full later.

The total white cell counts of beagles from Kennel I was high and also high in young dogs from Kennel S. (See Appendix page 44). These high figures were not considered to be due to breed variation and so the results from beagles housed in Kennel H were compared with the results from the other two kennels. The full results for beagles from Kennel H are given in the Appendix page 45. The following table shows the average results for total white cell counts per cu.m.m. from beagles in each kennel. Figures in brackets show number of dogs used.

	Kennel I.	Kennel S.	Kennel H.
3-6 months	17962	15216	11737 (2)
6 months to 9 months	14831	11649	10938 (5)
9 months to 1 year	18483	12200	9700 (1)
1-5 years	15134	8924	10545 (5)

These results show that beagles from Kennel H and adult beagles over six months from Kennel S have lower white counts than beagles from Kennel I and beagles under six months from

Kennel /

Kennel S. This suggests that beagles only have high white counts if environmental conditions stimulate white cell production, e.g. large kennels. To ensure that the lower results from Kennel H are true figures they were compared with the results obtained from a mixture of dogs of other breeds, of the same age, also living in Kennel H. This comparison of average total white cell counts per cu.m.m. yielded the following results:

Age	Beagles from Kennel H. 13 samples.	Dogs other than Beagles from Kennel H. (not greyhounds). 31 samples
3-6 months	11737	12814
6-9 months	10938	9762
9 months - 1 year	9700	10051
1-5 years	10545	11326

The results show clearly that beagles do not have higher white cell counts than other breeds of dogs living in the same environment; if anything they are slightly lower.

LABRADORS

A comparison of the blood pictures of pet labradors and working labradors was made to ascertain the labradors' blood picture. The fact that some labradors were working makes little difference to the blood picture as will be shown later (See working dogs) and this comparison is considered a fair one to ascertain breed variation. Four labradors from Kennel H were also compared.

The /

The average results obtained are as follows. The figures in brackets indicate the number of dogs used in each group.

	PET LABRADORS			WORKING LABRADORS			KENNEL H.		
Age	P.C.V. %	Hb. gms. per 100 mls.	W.B.C. per cu.m.m.	P.C.V. %	Hb. gms. per 100 mls.	W.B.C. per cu.m.m.	P.C.V. %	Hb. gms. per 100 mls.	W.B.C. per cu.m.m.
1 to 5 years	51.6	18.4	9978	51.1	18.1	10621	50	18.5	12900
	(14)	(14)	(14)	(15)	(15)	(15)	(2)	(2)	(2)
5 years and over	48.7	17.6	9721	47.8	17.3	8890	47	16.3	13725
	(14)	(14)	(14)	(15)	(15)	(15)	(2)	(2)	(2)

The number of dogs from Kennel H was small, only two dogs in each group, but apart from the white cell counts, they show little difference from the results obtained from the other two groups. The white cell counts are higher due to environmental factors.

These results show that there is little variation in any of the values discussed between labradors and beagles (in small kennels).

RESULTS FROM OTHER DOGS

A few adult dogs, of breeds other than the ones mentioned, were compared with the same breed of dog from another source to find out the probable values in these breeds. In the results which follow the figures in brackets again indicate the number of dogs used.

Mongrels age one to five years

PETS			KENNEL H.		
P.C.V. %	Hb. gms/100 mls.	W.B.C. per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	W.B.C. per cu.m.m.
51.7	18.4	11603	54	19.0	9787
(7)	(7)	(7)	(2)	(2)	(2)

Collies age one to five years

PETS			KENNEL H.		
P.C.V. %	Hb. gms/100 mls.	W.B.C. per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	W.B.C. per cu.m.m.
49.4	17.8	10215	48.5	17.8	9550
(5)	(5)	(5)	(1)	(1)	(1)

The above results cannot be considered as accurate values for the breeds involved as the numbers are too small. The slightly higher white cell counts in the pet dogs is due to one or two higher results in each group. These dogs all came from the town. These results show a close relationship to those from beagles and labradors and no breed variations can be seen.

CONCLUSIONS

No breed variations were seen with regard to red cell counts, packed cell volumes, haemoglobins and total white cell counts /

counts with the exception of greyhounds. Greyhounds have higher red cell counts, packed cellvolumes, haemoglobins and specific gravities than other dogs, and these high values are due to breed variation and not to environment or racing. Both very young and very old greyhounds showed higher values than other breeds of a similar age.

The total white cell counts in greyhounds tend to be low and the range of variation narrow. Poor enviromments do not seem to have any marked effect on them, although of course greyhounds with inflammatory conditions do show raised white cell counts.

Beagles kept in small kennels or as pets do not have raised total white cell counts as suggested by Irfan (1958).

No breed variations were noticed in the other estimations carried out on the blood samples.

VARIATIONS DUE TO WORK

No direct references of any sort have been found relating to this particular topic. There are several references on the effect of exercise, but this will be dealt with under the section dealing specifically with exercise.

PRESENT WORK

As greyhounds have already been shown to have high red cell counts, this part of the study was undertaken to see if similar results were obtained from working dogs of other breeds.

For the purposes of this section the term "work" indicates that the dogs are used either to do a specific job or used as racing dogs. This section therefore will deal with dogs used as gun-dogs or sheep dogs which are specially bred and trained for this work, and also with greyhounds raced by their owners for gain.

The results obtained from greyhounds and gun-dogs have already been given, but in this section the results were further sub-divided to compare the results from dogs working full-time and those working only part of the time. These two sets of results were then compared with results from non-working dogs of the same age and breed.

The results are dealt with in three sections - Greyhounds, Gun-dogs, and Sheep-dogs.

RESULTS /

RESULTS

Greyhounds

This breed have high red cell counts, haemoglobins, packed cell volumes and specific gravities. In the following table the average results from greyhounds which are racing and those which have not been racing for at least one month will be compared. The dogs are divided into age groups from the same kennel. The figures in brackets indicate the number of dogs used.

		"PETS"		Kennel C.	
Age		Racing	Not Racing	Racing	Not Racing
1-5 years	R.B.C. millions per cu.m.m.	7.428 (8)	7.280 (11)	7.695 (10)	7.455 (14)
	P.C.V. %	57.8 (8)	57.9 (11)	61.6 (10)	59.0 (14)
	Hb. gms/100 mls.	20.5 (8)	20.4 (11)	22.0 (10)	20.7 (14)

The dogs of one year of age in the racing group are generally in training and have been running in trials but not actually raced in competitions.

The results show that there is no significant difference between racing and non-racing greyhounds with regard to red cell counts, haemoglobin and packed cell volumes. No variations in other blood values were noted between the groups.

Gun-dogs

All the dogs in this section are labradors. All were bred from a strain of working labradors and not from show dogs. The amount of work done by each dog varied considerably and for this reason theyhave been put into two groups. The first consists of gun-dogs working every day of the week, and the second consists of gun-dogs which are worked once or twice a week and spend the rest of their time in kennels or private homes.

All thesamples were taken during the shooting season when the dogs were working.

The following results were obtained, and are compared with results from non-working pet labradors.

Age		Full-time working dogs	Part-time working dogs	Non-working pet labradors
1-5 years	R.B.C. millions per cu.m.m.	6.477 (6)	6.616 (9)	6.726 (14)
	Hb. gms/100 mls.	17.7 (6)	18.3 (9)	18.4 (14)
	P.C.V. %	50.1 (6)	51.7 (9)	51.6 (14)
5-9 years	R.B.C. millions per cu.m.m.	6.795 (5)	5.921 (9)	6.237 (9)
	Hb. gms/100 mls.	18.5 (5)	16.7 (9)	17.6 (9)
	P.C.V. %	51.3 (5)	46.1 (9)	48.6 (9)

These /

These results show clearly that work has little effect on the red cell picture. The full-time gun-dogs over five years of age had higher values than part-time dogs or pets but not markedly higher.

The reason for the values for full-time gun-dogs of one to five years of age being lower than either pets or part-time gun-dogs is their feeding. Five of the six dogs in this group came from one estate and the gamekeeper fed his dogs in the traditional Scottish way on porridge and maize meal. This deficiency diet is not ideal for the production of red cells and haemoglobin. All the dogs appeared healthy when sampled, but it is known that a vitamin B complex deficiency had occurred in these dogs on a previous occasion.

Sheep dogs

All these dogs are full-time working collies, on hill sheep farms. Only eight dogs were sampled and these results will be compared with the results from five pet collies living in the town. The results are as follows:

Age		Working Collies	Pet Collies
1-6 years	R.B.C. millions per cu.m.m.	6.315	6.237
	P.C.V. %	48.1	49.4
	Hb. gms/100 mls.	17.1	17.8

The results show no variations between the groups. Two of the working dogs W.2 and W.15 were fed on a good mixed diet and had high results. All the others lived on a diet of porridge and maize and their results were generally low, causing a reduction in the average values.

DISCUSSION OF RESULTS

The results from the greyhounds indicate that there are no significant changes in the red cell picture as a direct result of racing, but the values tend to be slightly higher in racing dogs than in non-racing dogs. Greyhound values are higher than those of other breeds and this is possibly due to the use of these dogs as racing dogs over many centuries. They have acquired a high red cell count as a direct result of racing and breeding, and this characteristic is now passed on from generation to generation.

The labrador is a young breed by comparison with the greyhound, and its red cell counts are not different from those of other breeds. Part-time work does not increase the red cell counts but full-time work possibly does. Any increase due to work can, however, be counteracted by poor feeding and this is shown clearly in the one to five year age group.

In a similar way working collies will only show raised red cell counts if their feeding is good. In the main Scottish shepherds feed their dogs very badly and grossly overwork them, and in such dogs the red cell counts will not be raised.

In a well fed greyhound the haemoglobin values are always high, but in a well fed working collie or labrador the haemoglobin values may not be raised.

No alteration was seen in any blood values, apart from the ones mentioned, as a direct result of work.

BLOOD PICTURE DURING THE OESTRUS CYCLE

REVIEW OF THE LITERATURE

In all the literature consulted there is not one account of the exact changes in the peripheral blood picture of bitches during the heat period.

Davis and Hulit (1949) noted a fall in the eosinophil counts in women during the menstrual period and gave the range of total white counts as 4,750 - 12,500 per cu.m.m. (normal 3,500 - 10,000 per cu.m.m.).

Canham (1930) described increased white counts at oestrus in cattle although he noted that some showed a reduction in their white counts. Any increase was due to rise in neutrophils. Irfan in his Ph.D. Thesis (1958) p.314 states that "several factors may cause minor physiological fluctuations in the leucocyte counts", and he mentions oestrus as one of these factors. However, he gives no results to confirm this statement.

OESTRUS CYCLE IN THE BITCH

Heat periods occur twice yearly in the bitch with an interval of approximately six months between each one.

The cycle is divided into four stages as follows: (Veterinary Obstetrics, Benesch and Wright, 1957, p. 21).

(a) Pro-oestrus

This lasts 9-10 days (range 4-14 days) during which time there is a flow of blood from the vulva.

(b) /

(b) Oestrus

Discharge ceases and this stage lasts for nine days (range 5-12 days). Mating is accepted during this period.

(c) Metoestrus

This lasts for eight weeks and may end in false pregnancy.

(d) Anoestrus

Period from the end of metoestrus to the beginning of the next pro-oestrus period.

PRESENT WORK

Ten bitches were used and twelve oestrus periods investigated. The ten bitches used for this study were obtained from several sources. Four were the property of Kennel H, two were from Kennel S and four were pets.

Five oestrus periods were studied without the use of controls. Five oestrus periods were investigated using four controls (two bitches were in season at the same time and the same control was used for both). In addition two of the controls were also studied during their own oestrus periods, making a total of seven oestrus periods studied using four controls.

Where possible samples were taken from each bitch every second day, either until the period of pro-oestrus and oestrus had been completed, or until the bitch was mated. In the latter cases it was not considered desirable to continue sampling in case the owner blamed failure of the bitch to conceive on interference with her during the time of conception.

The /

The onset of oestrus is difficult to detect unless a close watch is kept on the bitch, and in all the cases studied the day of onset of oestrus was known exactly. In no case was a bitch used in this work when the date of onset was uncertain.

Sampling started when the bitch first showed enlargement of the vulva and a blood-stained discharge. It was impossible to ascertain exactly when a bitch was to come in season and therefore it was not possible to take samples the day before or even a few days before pro-oestrus commenced.

Throughout the period of sampling the bitch's routine was kept as near normal as possible.

RESULTS

As the bitches studied came from different sources it may appear that the control and the bitch in oestrus came from different kennels. This is often true, as it was impossible in most instances to obtain two bitches from the same place, so I had to "borrow" one of the bitches from the owner. In all cases the two bitches were kept in the same kennel - usually in the College Kennels - under an identical system of management during the oestrus period.

The results obtained showed that only slight variations occurred in the blood picture during oestrus, in many cases less than the maximum daily variations in normal dogs. They were not considered to be of any statistical significance. Complicated statistical evaluation was not carried out as the results obtained did not warrant it.

Red /

Red Cells, Haemoglobin and Packed Cell Volume

Six bitches showed a slight fall in these values (never exceeding 10%) but in three of these they rose again after the eighth day. Six bitches showed no fall; two controls showed falls (less than 5%) and two showed no alteration. In no case did the red count, haemoglobin or packed cell volume fall below the normal range.

Normal red cell replacement coped with the loss of blood from the vulva.

In no case was there a rise in normoblasts. Bitch H.14 showed a very high normoblast count, up to 9,000 per cu.m.m., but this particular bitch has shown abnormal counts since the first sampling at four months of age and these have persisted over many months. Her red counts have always been high and she has never showed signs of ill health over the last two years, apart from metritis after whelping.

The reticulocyte counts rose in five bitches. Controls showed no rise. In two cases the reticulocytes exceeded 1% of the red cell count showing that active red cell replacement had been taking place.

Platelets

Seven bitches showed an overall increase in platelets; two showed no change; one fell, and in one there was persistent clumping, making counting inaccurate.

Two of the controls showed an overall increase and two no change. There appears to be a slight increase in platelet production /

production, although the rise is erratic, and occasionally absent in some dogs. This is due to the increased demand for platelets due to blood loss during oestrus. In part the increase may also have been due to the repeated bleedings as two controls also showed signs of an increase in platelet production. This is shown in the table.

Table showing changes in platelets thousands per cu.m.m. during oestrus

Day of Pro-oestrus	Five bitches in oestrus. No controls	Seven bitches in Oestrus	Four Controls
1 - 3	374	364	272
4 - 6	364	393	305
7 - 10	---	383	289

Table showing average Platelet counts thousands per cu.m.m. from bitches P.23, H.14, P.24 and P.77 at first sampling, and on seventeenth to nineteenth day of oestrus, together with their controls.

1st Sampling	17th-19th day of Oestrus	Controls Before	Controls After
266	305	239	265

The rise in platelet counts is small. It will be noted, however, that the average platelet count for bitches in oestrus is higher than that of the bitches used as controls (See first table), and possibly some rise in platelets occurs before clinical signs of oestrus appear. The figures in the first table are higher than the average normal values for dogs.

Total White Counts and Differentials

A total of eight bitches showed a rise in the total white counts. Of the twelve bitches examined during oestrus, eight had maximum white counts during the 6th-8th days of the heat period. In the other four the white counts were at a maximum during the 1st-4th day of the heat period.

There is a rise in the white cell count during the period of pro-oestrus and oestrus. This rise is variable in extent and duration but is more likely to occur during the pro-oestrus period. In four cases the white count never exceeded 14,000 cells per cu.m.m.

During true oestrus (after tenth day) the white cell counts were usually within the range of normality.

Table showing average total white cell counts per cu.m.m. during oestrus

Day of Oestrus	Five Bitches in Oestrus No Controls	Seven Bitches in Oestrus	Four Controls
1 - 3	14635	12706	9533
4 - 6	14058	14602	12184
7 - 10	...	13168	11483

These results show that the bitches in oestrus have higher average white cell counts than the controls. The reason for the higher control result in the 4th-6th days group is that control P.77 had a count of 17,750 per cu.m.m. which raised the average result. The cause for this is unknown. After the tenth day of oestrus /

oestrus the white cell counts fell in most cases back to normal.

Bitch P.23 never showed any rise in her white cell count during the pro-oestrus period.

Bitches P.77 and S.24, which were bled as controls and also during their own oestrus showed the following average white cells per cu.m.m. results.

Day of Oestrus	P.77 As control	P.77 Pro-oestrus	S.24 As control	S.24 Pro-oestrus	Second Pro-oestrus
1 - 3	----	13600	10625	11315	14700
4 - 6	* 15062	14800	13612	13835	14975
7 - 10	12512	16575	12662	16150	15175

* This result is high due to the high white cell count described above.

These results show clearly that in these bitches the total white cell counts were raised during pro-oestrus. This rise is of clinical but not statistical significance.

The rise in the total white cell counts is due mainly to a rise in neutrophils but no shift to the left was observed. The lymphocyte absolute figures remained the same during this period; monocytes were slightly raised, but eosinophils and miscellaneous cells appeared to be unaffected.

The variations in cell type during the complete heat period is shown here:

	Average Mean White Cell count per cu.m.m.	Average Mean Neutrophil count per cu.m.m.	Average Mean Lymphocyte count per cu.m.m.	Average Mean Monocyte count per cu.m.m.	Average Mean Eosinophil count per cu.m.m.	Average Mean Misc. cell count per cu.m.m.
7 bitches in oestrus	13,284	8,992 67.7%	2,997 22.6%	548 4.1%	688 5.1%	58 0.4%
Four Controls	10,977	6,929 63.1%	3,026 27.6%	405 3.6%	555 5.0%	61 0.5%
Five bitches in oestrus. No controls	14,201	9,837 69.3%	3,163 22.3%	699 4.9%	473 3.3%	27 0.1%

Both the absolute and percentage figures for neutrophils are higher during oestrus than the figures for the controls. The lymphocyte percentage figures fell during oestrus but there was no alteration in the absolute numbers of circulating lymphocytes during oestrus. The only significant alteration is the increase in lobulated neutrophils during oestrus. This rise may be confused with pathological neutrophilia, in pet dogs.

Other changes during the Heat Period

Specific Gravity

No significant changes occurred, the specific gravity tending to follow the red cell counts. The highest value seen was 1.0685, and the lowest 1.051. The values did not vary by more than /

than 0.007 between the first and the last day of sampling in any one bitch.

Sedimentation Rate

In almost all cases the reading after one hour was nil. In three bitches the rate rose to $\frac{1}{2}$ m.m. and in one case to $1\frac{1}{2}$ m.m. after one hour. None of these rates are of any clinical significance.

In three bitches P.24, S.2 and R.D.V.C.1 (control) the sedimentation rates were high, ranging from 2 - 6 m.m. per hour. All these bitches were over seven years of age and the increased rate is probably due to their age and not to the fact that they were in oestrus. In all three cases the rate was high both at the beginning and the end of the sampling period.

Corpuscular Values

No changes which could be correlated with the oestrus cycle were observed.

CONCLUSIONS

The red cell counts and related estimations may fall slightly during oestrus but this change is insignificant. The platelet counts tend to be above the normal average (350-400,000 per cu.m.m.) during oestrus but this change varies considerably from bitch to bitch.

The total white cell counts in most cases were higher during the pro-oestrus period and then fell to normal after this. The /

The average total white counts for all twelve bitches during the fourth to sixth day of pro-oestrus was 14375 per cu.m.m. In one bitch no rise occurred at all and in three bitches the rise was slight. The length of time during which the total white cell count was raised varied considerably from one bitch to another.

This increase in white cells is due to an increase in mature neutrophils.

Although the white cell counts tend to be higher during oestrus they do not vary markedly, and in several cases do not vary more than would be expected from the daily variations of normal dogs.

No other changes associated with oestrus were seen.

THE BLOOD PICTURE DURING PREGNANCY

INTRODUCTION

Gestation in the bitch lasts for a period of nine weeks. Benesch and Wright (1957) state that "the gravid uterus and its contents cause no appreciable increase in bodyweight during the first five weeks". From this point it rapidly increases according to the number of foetuses.

Abdominal distension and enlargement of the mammary glands occur during pregnancy but similar changes are also seen in false pregnancy. Abdominal distension may also be caused by ascites, enlargement of the liver or spleen, pyometra or neoplasia of abdominal organs. All these conditions may hinder the diagnosis of pregnancy or be confused with it.

The main methods of pregnancy diagnosis in the bitch are abdominal palpation and radiographic examination. Pregnancy can only be diagnosed with any degree of accuracy using X-rays during the final week of pregnancy. The accuracy with which pregnancy can be diagnosed by abdominal palpation will depend on whether the bitch is overweight, how many foetuses are present, the stage of pregnancy and whether she resents palpation or not.

In cases of fat bitches, carrying only one or two puppies, it is often virtually impossible to make an accurate pregnancy diagnosis until the eighth or ninth week of gestation. As many breeders /

breeders wish to know if a bitch is pregnant as early as possible, examinations of the blood from pregnant bitches were performed to assess whether this method could be used as an accurate and early method of pregnancy diagnosis, especially in cases where abdominal palpation proved difficult.

REVIEW OF THE LITERATURE

The literature related to the blood picture of bitches during pregnancy is rather scanty and in most cases the work has not been done as a detailed study.

As early as 1904 Thompson described the changes which occurred in the blood of women during pregnancy. He found that the erythrocytes fell until the sixth month of pregnancy, but that the total counts were high at the beginning and also at the end of pregnancy. The haemoglobin values and specific gravities followed a similar pattern.

The total white cell counts varied very little during pregnancy. He gives average differential counts but does not state how the differentials varied throughout pregnancy.

Burnett and Traum (1905) gave a few results for one bitch which was pregnant and also took three samples after whelping. They made no attempt to follow the blood picture changes during the complete period of pregnancy. Their results are as follows:

Stage of Pregnancy	Erythrocytes per cu.m.m.	Hb. %	Leucocytes per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Neut. per cu.m.m.	Eosin. per cu.m.m.
5 weeks	5,742,000	81	17,888	3,040	715	13,200	894
6 wks. 6 days	5,474,000	80	23,666	3,118	1,520	18,290	807
7 wks. 6 days	4,923,000		23,240	3,093	2,235	17,300	628
whelped one day	4,356,000		19,166	1,916	1,244	15,570	421
whelped 22 days	4,404,000		12,400	2,292	1,849	8,210	50
whelped 46 days	6,426,000	91	11,296	1,468	1,012	8,100	71

Percentage figures were also given for the differential results.

Wells and Sutton (1915) took samples from eight bitches which had whelped between 2 and 151 days previously. They stated that the red cell counts were low just after parturition (3.5 - 4.5 million per cu.m.m.) and that these counts rose slowly after this.

Canham (1950) in his work on the blood of cattle observed that the red cell counts fell during pregnancy but were high at the time of parturition.

Scarborough (1931) states that Welcker found that there was a slight diminution in the erythrocyte count during pregnancy. He also says that Corsy and Meyer have both found a slight increase in lymphocytes and a corresponding decrease in polymorphonuclears in pregnant dogs. He omits to give reference details for these three articles.

Simms /

Simms (1940) found that the sedimentation rates increased during pregnancy to 1 - 52 m.m. per hour with an average of 15 m.m. per hour.

Crafts (1948) did work on the effects of oestrogens on the bone marrows of adult female dogs. Blood pictures were obtained before and after treatment with oestrogens. He found that the initial reaction was a great increase in the neutrophilic elements of the bone marrow. These were released into the blood stream and resulted in a rise in the total white cell count. After this there was a fall due to the toxicity of oestrogens on the bone marrow. He was unable to produce similar changes in humans or in monkeys. (See also references on oestrogens in sections on pyometra and mammary tumours).

Sunderman and Boerner (1950) give considerable details on the changes during pregnancy in women. They are as follows:-

The blood volume increases by up to 23%. The packed cell volume falls by 14%, but the red cells numbers increase although this is masked by the increased blood volume and in fact the number of cells appear to decrease. Haemoglobin values follow the red cell deviations. The sedimentation rates increase progressively.

White cell counts increase very slightly especially the neutrophils. During labour the white counts are very high (up to 30,000 per cu.m.m.). The platelets show no specific changes and the clotting times are normal.

Albritten /

Albritton (1955) states that the sedimentation rates for bitches 11-56 days pregnant is 1-52 m.m. per hour.

Anderson and Gee (1958) using Beagles followed the changes in the blood during pregnancy.

	Gestation				Term	Lactation		
Time in weeks -	2	4	6	8	0	2	4	6
Erythrocytes 10^6	8.85	7.48	6.73	6.26	4.53	5.13	5.65	6.15
Packed cell volume	53	47	44	37	32	34	38	42
Haemoglobin grms/100 mls.	19.6	16.4	14.7	13.8	11	11.7	12.8	13.4
Sedimentation Rate m.m. per hour	0.6	11	31	14	12	14	14	13
Leucocytes 10^3	12	12	15.7	19	18.9	16.9	17.1	15.9

Meilke (1961) did blood studies in 18 cats during pregnancy. His results showed that the red cell counts and haemoglobin values fell. The leucocytes rose from an average of 9,000 per cu.m.m. in normal cats to an average of 14,000 in pregnant animals, the rise being due to an increase in neutrophils. The sedimentation rates were markedly increased.

Sasaki (1961) showed that the sedimentation rates increased during pregnancy in dogs and that rouleaux formation was seen in blood smears from such animals.

Reda and Hathout (1951) say that the total erythrocyte count /

count and haemoglobin values tend to decrease late in gestation. A neutrophilic leucocytosis is seen during the last month of pregnancy and lymphocytes decreased in proportion to the increase in neutrophils. There is a slight eosinophilia during the period. This work was done on sheep.

In cattle at parturition the total leucocyte counts are increased due mainly to a rise in neutrophils, and eosinophils are decreased. The total leucocyte count may fall or increase by the first day after parturition and if it decreases this is due to a reduction of both neutrophils and lymphocytes. These results were obtained by Ferguson, Irwin and Beach (1941), and Straub, Schalm, Hughes and Theilen (1959).

DISCUSSION OF LITERATURE REVIEWED

The articles dealing with the blood changes in bitches during pregnancy are very incomplete, the majority of authors dealing only with one or two aspects of the problem. Anderson and Gee (1958) did the most comprehensive work. Their bleedings were only performed at fortnightly intervals and no indication is given of the number of bitches used in the work. They did not use controls for their work.

The remaining articles show that changes occur in the blood of other species. In the human the changes were less marked than in the dog.

The articles reviewed indicate that the erythrocyte counts, packed /

packed cell volumes, and haemoglobin values all fall during pregnancy. The total white cell count rises, mainly due to an increase in neutrophils, and that sedimentation rates also rise. The extent and duration of these changes is not shown clearly and no attempt has been made to assess the changes occurring in false pregnancies, abnormal pregnancies where mummification occurs, or in bitches which are mated but apparently fail to conceive.

PRESENT WORK ON THE BLOOD PICTURE DURING PREGNANCY

Bitches were obtained from most of the sources already described, and their routine was kept as near normal as possible throughout the period of pregnancy. The only changes were in the diet. The amount and quality of the food was increased as parturition approached but this is the normal practice with bitches in whelp.

Blood samples were taken at weekly intervals until parturition, and after parturition samples were taken at varying intervals for a period of several weeks.

In a few cases the bitches were mated more than once during oestrus. If a bitch was mated on the 10th, 12th and 14th day of the month the pregnancy was taken as commencing on the 12th.

SOURCES AND NUMBER OF BITCHES USED

The bitches were obtained from Kennels H, R.D.V.C. and S. A considerable number of pets were also used. A total of thirty-five cases of pregnancy or possible pregnancy were studied.

Six /

Six bitches were followed through pregnancy together with four normal controls from the same kennels, and seven bitches were studied without controls. One bitch in which her puppies subsequently were mummified, and one bitch which was mated but failed to whelp were examined. A further twenty mated bitches were bled to assess the value of the blood picture as an aid to pregnancy diagnosis.

RESULTS AND DISCUSSION

The average results are in the Appendix pages 45a to 47.

The results obtained during this work showed that marked changes occurred in the blood picture and that these changes can be correlated with the stage of pregnancy.

The bitches whose pregnancies were studied throughout the nine weeks, are divided into two groups. The first group consists of six pregnant bitches and the four controls run with them, and the second group of seven pregnant bitches with whom no controls were used.

It was found that the mean average results from each group of pregnant bitches were very similar. All changes in the blood picture occurred at specific times during gestation and were seen in both groups.

The mean average results are shown graphically in the text and the tables of average results are given in the Appendix, pages 45a to 47.

Red /

Average values from six pregnant bitches and their controls together with the values from seven other pregnant bitches.

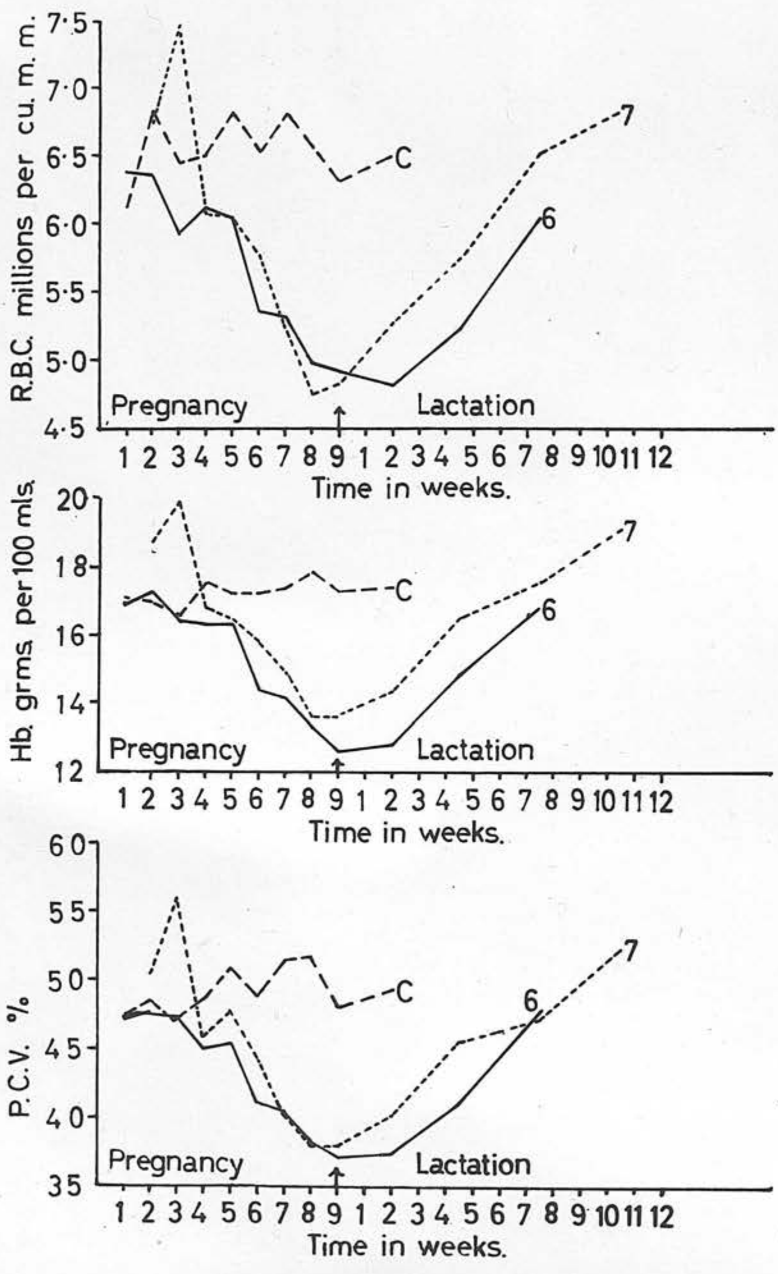


FIG. 5

Red cells, Haemoglobin and Packed Cell Volumes

In all pregnant bitches these three values fell throughout pregnancy. The values fluctuated during the first four weeks of pregnancy but thereafter decreased. (See Fig. 5). All three average values fell to the minimal normal limits by the end of the sixth week of pregnancy. In seven out of ten cases examined during the last week of pregnancy the red cell counts were below five million per cu.m.m., the packed cell volume below 40%, and the haemoglobin values 14 grms. per 100 mls. or less.

The apparent reduction in red cells varied from one case to another. The maximum fall was 3.1 million per cu.m.m. (7.4-4.3 millions per cu.m.m.), and the minimum 0.7 millions per cu.m.m. (5.5-4.8 millions per cu.m.m.).

The greatest reductions were seen in cases of young animals whose level of nutrition was not increased sufficiently as pregnancy progressed. Even if the level of nutrition ishigh there is always an apparent fall in the red cell counts. This "fall" is probably partly due to an increase in blood volume rather than an absolute fall in the number of red blood cells. This was not proved, however, as blood volume studies were not carried out, but Sunderman and Boerner (1950) showed blood volume increases in pregnant women. The four control bitches showed no significant decrease in red cell counts, packed cell volumes or haemoglobin values.

After /

After whelping, the red cell counts, packed cell volumes and haemoglobin rose slightly but it usually took three weeks before any appreciable increases occurred. From the third week after parturition onwards, all values increased steadily, and they returned to their original levels approximately nine weeks after parturition.

The time it took for the levels to get back to normal varied from one bitch to another, and depended on the feeding and the number of puppies being suckled. In general, bitches with large litters took a little longer toreturn to normal than did bitches with small litters.

These results confirm the work done on dogs by Burnett and Traum (1905), Wells and Sutton (1915) and Anderson and Gee (1958). They are also similar to the results of Canham (1930) and Reda and Hathout (1951) whose work was done in cattle, Sunderman and Boerner (1950) working with humans, and Meilke (1961) working with cats.

Erythrocyte Regeneration

The number of normoblasts and reticulocytes showed alterations. There was little or no variation in numbers until about the 45th day of gestation. After this time the number of reticulocytes increased in all pregnant bitches reading a maximum value just prior to parturition. The majority of bitches had reticulocyte /

Average values from six pregnant bitches and their controls together with the values from seven other pregnant bitches.

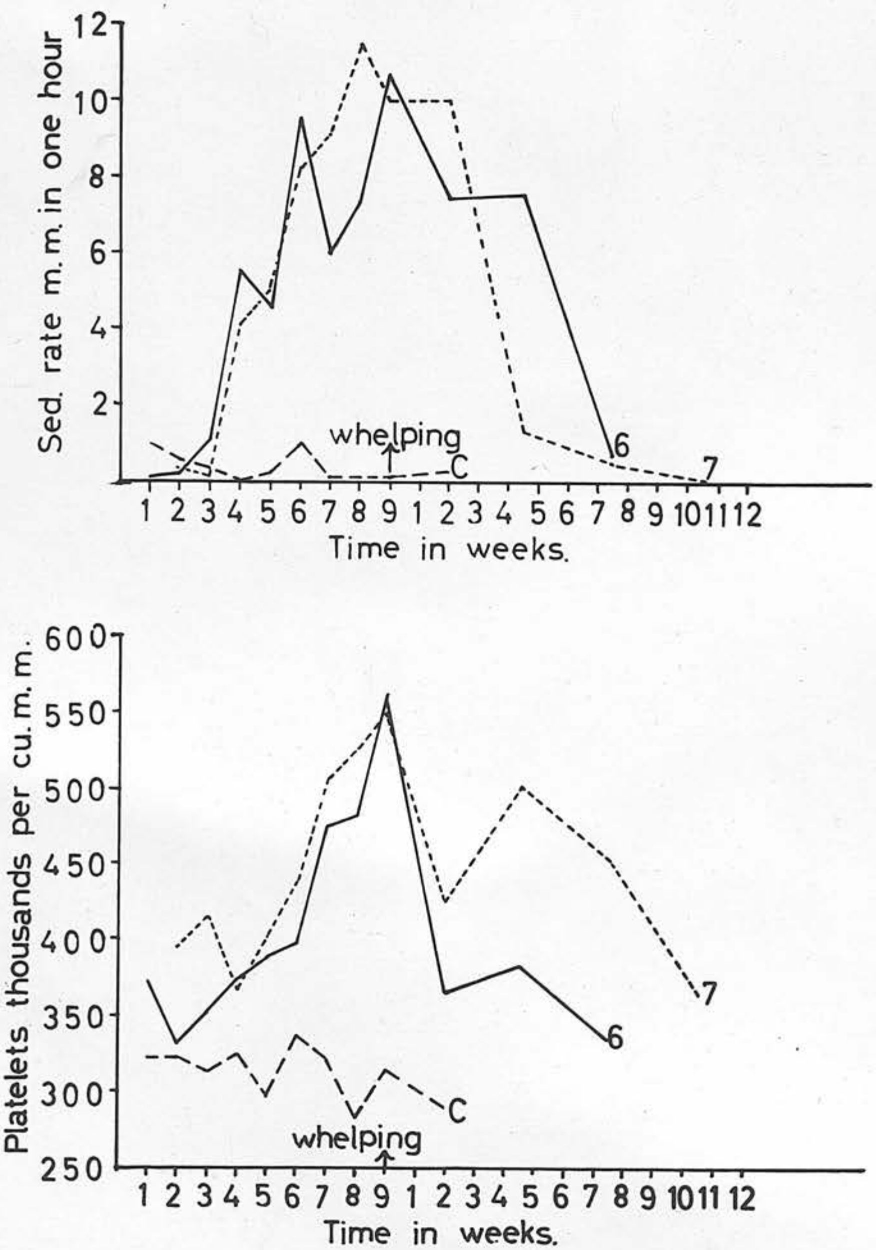


FIG. 6

reticulocyte counts of approximately 1 - 2% just prior to whelping; in four cases there were 3%, and in one case over 5% of reticulocytes. In two cases there was an increase in the number of normoblasts just prior to parturition, but none of the other bitches showed any alterations. The maximum rise was in case H.1 when normoblasts were 1,075 per cu.m.m. on the fifty-second day of gestation. There were no alterations in the numbers of normoblasts or reticulocytes in the four bitches used as controls.

After whelping, the number of reticulocytes fell to a normal value of between 0.1% and 0.5%. The length of time taken to reach normal values varied, but was between one and four weeks.

Sedimentation Rates

These increased steadily throughoutpregnancy, maximum values occurring during the last three weeks of pregnancy. (Fig.6). The rate of increase varied and in some cases the increase was slight and not outside the range of normal limits. The sedimenta-:tion rates were not constant and tended to fluctuate considerably even when the overall average was higher than normal. The mean average values for sedimentation rates during pregnancy were markedly higher than those of the controls.

The plasma zone was generally cloudy, especially in cases where gestation was advanced, due to increased numbers of reticulocytes and also cholesterol and fat. In most cases the plasma zone showed a clear area at the top and a larger opaque or pink area below.

Following /

Following parturition the sedimentation rates remained high for several weeks. The average values had returned to normal after six weeks. These results are in line with those of Simms (1940), Anderson and Gee (1958) and Sesaki (1961) who all recorded increased sedimentation rates during pregnancy.

Platelets

Generally there was some fluctuation in platelet numbers for the first three or four weeks of pregnancy and then the numbers gradually increased until the sixth or seventh week. At this point the increases became more marked and just before parturition the platelets numbered 500,000 per cu.m.m. or more. (Fig. 6). Nine of the thirteen bitches studied had counts in excess of 500,000 per cu.m.m. prior to parturition.

The rise in platelets was not uniform in all cases. H.33, the only exception to be seen, showed a decreasing platelet count just prior to parturition and never had a count exceeding 375,000 per cu.m.m. (Her counts in fact fell from 375,000 - 215,000 per cu.m.m.).

The maximum rise seen in platelet numbers was 335,000 per cu.m.m. (390,000 - 725,000 per cu.m.m.).

The platelet counts decreased sharply immediately after whelping. This is due to the great demand for platelets during whelping to control haemorrhage. This fall is seen clearly in bitches S.3 and S.4 (Appendix pages 48 and 49). After three or four days the counts rose again and in some cases soon exceeded the /

the numbers before parturition. The counts then started to decrease, but often very erratically, and the average platelet counts are above 350,000 per cu.m.m. nine to twelve weeks after parturition.

Although the results are not given here, a few bitches which have been examined regularly had elevated platelet counts which persisted for several months and in two cases the counts remained high following pregnancy until the next oestrus.

No references were found quoting the values for platelet counts during pregnancy in dogs, but these results differ from those o of Sunderman and Boerner (1950) who found no change in the platelet counts of women during pregnancy.

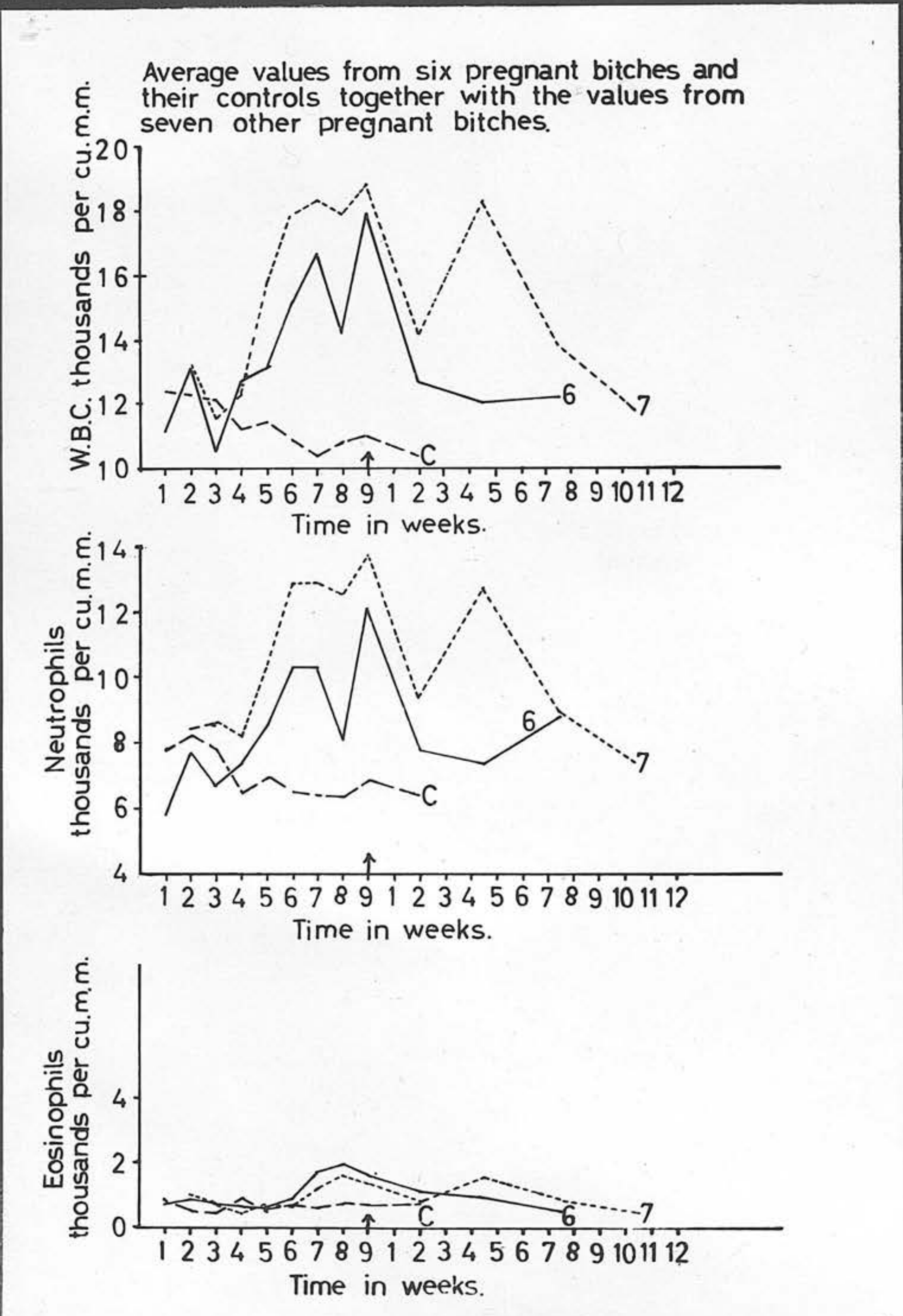
Corpuscular Values

No marked significant changes were observed in the corpuscular values during gestation. The only change is a slight increase in the M.C.V. during the last two weeks of pregnancy, which is not statistically significant.

Specific Gravities

These followed the same trend as the red cell counts, fluctuating for the first few weeks of pregnancy and then falling markedly as parturition approached. The greatest fall was in H.33 when the specific gravity fell during pregnancy from 1.0665 to 1.047.

The mean specific gravity values for the thirteen pregnant bitches studied during the second, sixth and final weeks of pregnancy /



Average values from six pregnant bitches and their controls together with the values from seven other pregnant bitches.
W.B.C. thousands per cu.m.m.
20
18
16
14
12
10
6
7
C
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 10 11 12
Time in weeks.
Neutrophils thousands per cu.m.m.
14
12
10
8
6
4
6
7
C
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 10 11 12
Time in weeks.
Eosinophils thousands per cu.m.m.
4
2
0
C
6
7
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 10 11 12
Time in weeks.

FIG. 7

pregnancy are given below:

	2nd week	6th week	final week
Specific Gravity	1.063	1.059	1.051

The specific gravities returned to normal at the same time as the red cell counts and packed cell volumes.

Total White Cell Counts

Considerable changes occurred in the white cell counts. During the second week of pregnancy there was a slight rise in numbers but this fell again by the third week. From the fourth week onwards the numbers rose steadily, reaching a peak during the seventh week. After this the numbers declined until parturition. (Fig. 7). A few hours prior to, and during parturition, there was a sharp rise in the total white count, mainly due to an increase in mature neutrophils. (See S.3 and S.4, Appendix pages 48 and 49). This rise is the cause of the apparent increase in white cells during the last week of pregnancy shown on the graph. (Fig.7).

The nature of the white cell increase is very variable during the first three or four weeks of pregnancy. In some cases lobulated neutrophils and in a few other cases lymphocytes made up the extra cells. The main persistent rise, however, until the seventh week was due to an increased number of lobulated neutrophils (Fig.7). No shift to the left was observed. Lymphocytes showed no significant variations during or after pregnancy.

During /

During the final three weeks of pregnancy the number of eosinophils was often increased (Fig.7), although the degree of increase varied from bitch to bitch. In one case (H.28) the eosinophils reached 7,483 per cu.m.m. on the fifty-second day of pregnancy. This bitch had no worm burden and was in excellent health throughout pregnancy. She was delivered by caesarian section on the sixty-second day - all the pups were alive and were reared. In a few cases no increase in the number of eosinophils was seen, and in two cases the eosinophil numbers fell.

Monocytes did not vary except for a rise during the fifth week of pregnancy. This rise was seen in both groups.

The "miscellaneous cells" shown in the tables consisted mainly of proplasmocytes , proplasmoblasts and lymphoblasts. The number of proplasmocytes appeared to be increased during pregnancy in seven bitches although the rise was very slight. Proplasmocytes were seen in all but three of the thirteen cases of pregnancy studied.

The number of neutrophils with "female" projections varied very little during gestation; there was a slight fall but not an almost complete reduction as is seen in uterine conditions with which pregnancy may be confused. (See Pyometra). In the first week there was an average of 10.8% of neutrophils with female projections, in the sixth week 7.7% and in the final week 6.7%.

After parturition the total white cell counts fluctuated but in many cases showed a rise between the third and sixth week. After the sixth week the counts fell returning to normal about nine weeks after whelping.

These /

These results confirm those of Wells and Sutton (1915) and Anderson and Gee (1958) with regard to the increased total white cell counts.

THE BLOOD PICTURE DURING LACTATION

Average results are given in the Appendix pages 45a - 47.

The blood picture is exactly the reverse of that seen during pregnancy. Shortly after parturition the red cell counts, haemoglobin, packed cell volumes and specific gravities are low, the total white cell counts, platelet counts and sedimentation rates are increased above normal. All these values slowly return to normal provided the bitch remains in good health.

The total white cell and differential white cell counts return to normal a few days after whelping, but fluctuations were seen, and the total count was above normal in five out of eight cases, twenty to thirty days after whelping. The red cell counts, haemoglobin, packed cell volumes and sedimentation rates had returned to normal six to eight weeks after whelping. In bitches without puppies suckling, these values returned to normal more rapidly than if suckling were taking place.

MATING FOLLOWED BY A FALSE PREGNANCY

One bitch S.2 was followed after mating and exhibited a falling red cell count until the twenty-fourth day, but after this it fluctuated and did not fall steadily as in a true pregnancy. (Appendix page 50). The sedimentation rate was increased until the /

the twenty-fourth day after mating and then it fell towards its original value. The white cell counts fluctuated reaching a maximum of 14,225 on the fortieth day. The platelet counts rose at first but declined after the thirty-second day.

This animal failed to show the typical complete changes seen in pregnancy but partial changes did occur and on the fortieth day the blood picture was similar to that of a pregnant bitch. It may have been that this bitch conceived but the pups died at an early stage, as bitches with false pregnancies which have been sampled have all had normal blood pictures. (See False Pregnancy)

This is one instance showing that the blood picture is not a reliable aid to pregnancy diagnosis in old bitches.

PREGNANCY DIAGNOSIS

As changes occur in the blood picture during pregnancy it was considered possible to diagnose pregnancy from the blood picture and for this purpose mated bitches were sampled. The results can be seen in the Appendix pages 51-52.

RESULTS AND DISCUSSION

It rapidly became obvious that the blood picture was a very unreliable method of pregnancy diagnosis before the end of the fifth week of pregnancy. From the fifth to the seventh week, however, it was quite reliable.

From the results already obtained in the earlier work on pregnancy a set of figures was elucidated and these were used as a basis from which a diagnosis of pregnancy could be ebtablished. These figures are as follows:

Red Cell Count per cu.m.m.	less than 6,000,000
Packed Cell Volume %	less than 45
Haemoglobin grms/100 mls.	less than 16
Sedimentation Rate m.m. per hour	1 or more
White Cell Count per cu.m.m.	13 - 30,000
Neutrophils per cu.m.m.	9,000 or more - no shift to the left
Platelets per cu.m.m.	400,000 or more.

These figures are for bitches between the 35th and 50th day of pregnancy.

When the bitches already discussed are used as examples we find the following. At the thirty-fifth to the fortieth day of pregnancy /

pregnancy eleven pregnant bitches were examined and of these six could be diagnosed as pregnant if the table just given is used as a guide. Of the remaining bitches, two had red cell counts above six million per cu.m.m., four had platelet counts below 400,000 per cu. m.m. and one of these also had a low total white cell count. Of the four controls, two could have been confused with possible pregnancy.

During the forty-fifth to the fifty-second day of pregnancy twelve bitches and four controls were examined. Of these eleven bitches could be diagnosed as pregnant from the table although one had a slightly higher packed cell volume and another a lower platelet count than given in the table. The other values conformed to the table. One pregnant bitch did not conform to the table. All four controls showed results which could not be confused with pregnancy.

In order to confirm the above results, twenty mated bitches were sampled once or twice only, to assess if the results obtained could be used to aid pregnancy diagnosis. The results are shown in the table, Appendix page 53. A partial positive result is one where four or five estimations correspond with the table.

These results show that it is not possible to diagnose pregnancy from a blood picture before the thirty-fifth day of pregnancy. Between the thirty-fifth and fiftieth day the chances of diagnosis are good. Of the seven pregnant bitches examined during this period five gave positive results, one a partial positive and one a negative result. Unfortunately the negative result was given by a bitch with only one pup, the type of case in which pregnancy diagnosis is difficult by other means.

After /

After the fiftieth day the results are not so good,as the white cell counts tended to fall and only partial positive results were obtained.

In bitches which were not pregnant,partial positive results were seen before the thirty-fifth day. Two partial positives were also seen between the forty-first and fiftieth days - one of these dogs had a cystic endometritis (P.54) and the other had been in a fight and received severe bite wounds (H.25). (Appendix pages 51 and 52).

If the results from all the pregnant and non-pregnant bitches examined between the thirty-fifth and fiftieth day of pregnancy are examined we find the following:

Number of Pregnant Bitches	Number diagnosed as Positive	Number diagnosed as partial positive	Number diagnosed as Negative	Number of non-pregnant bitches	Number diagnosed as partially Positive
19	16	1	2	11	2

No non-pregnant bitch was diagnosed as a complete positive.

It is consddered that this method of pregnancy diagnosis will prove reasonably successful between the thirty-fifth and fiftieth day of pregnancy. It is not as successful in bitches carrying only one or two puppies as it is in bitches with larger numbers of pups. From this viewpoint it may not be as useful as had been hoped as it is in this type of bitch that pregnancy diagnosis is difficult.

The /

The method is unreliable before the thirty-fifth and after the fiftieth day. In the latter case this is due to the fall in white cells which often occurs. The method is not reliable in dogs which are diseased, old, or have been injured. (See H.25 and P.54). (Appendix pages 51 and 52).

This method would prove useful in a doubtful case of pregnancy if the results obtained were all outside the values given in the table, e.g. a bitch mated forty-five days previously with a red cell count of 7,000,000 per cu.m.m., packed cell volume of 50 and a white count of less than 10,000 per cu.m.m. would not be pregnant. In such cases more reliance can be placed on the values for red cell counts, packed cell volumes, and haemoglobins than on white cell or platelet counts. If the total white cell counts are above 30,000 per cu.m.m. some abnormality should be suspected.

This method of pregnancy diagnosis is of course more accurate if the blood values for a particular bitch are known prior to, or just after, the commencement of pregnancy.

The best time to sample dogs for pregnancy diagnosis by this method would be the fortieth to fiftieth day after mating.

POSSIBLE PREGNANCY FOLLOWED BY RE-ABSORPTION OF FOETUSES

The results of this individual animal are given in the Appendix page 54.

This bitch had a previous history of being mated, and, appearing to be pregnant, but during the eighth or ninth week the distended abdomen slowly subsided and no pups were produced.

During the pregnancy recorded, the bitch appeared perfectly normal and healthy throughout the whole period. The abdomen became distended by the sixth week and mammary development took place. At the eight and a half week stage a dirty vulval discharge was observed which persisted for three days and then cleared up. The bitch failed to whelp and the abdomen subsided and was normal after ten weeks.

RESULTS

In this case the blood picture showed changes which were in some ways similar to those seen in pregnancy. The red cell counts were low on the thirty-seventh day but failed to fall steadily after this. The haemoglobin and packed cell volumes followed the same pattern. The sedimentation rate was high and so was the platelet count although this latter started to fall after the forty-fourth day. The total white cell and differential white cell counts rose as the apparent pregnancy proceeded but by the forty-fourth day had reached 47,000 per cu.m.m., a value much in excess of that seen in normal pregnancies. The total count rose to a maximum of 56,000 per cu.m.m. on the fifty-eighth day. This increase was due to a rise /

rise in neutrophils, and a shift to the left was seen. As the abdomen decreased in size and the vaginal discharge ceased, the white count fell very rapidly. The reason for so rapid a decline is unknown.

DISCUSSION

It seems probable that mummification and re-absorption of the foetuses occurred during the fifth or sixth week of pregnancy. This would account for the blood changes seen and also the clinical signs of pregnancy. This was not a true case of false pregnancy in which blood changes do not occur. (See section on False Pregnancy).

The blood picture seen in the later stages is identical with that seen in pyometra but this could not have been such a case as the bitch has been in season three times since, at regular intervals. At the third oestrus, following the re-absorption, it was decided to mate the bitch again, and to continue the pregnancy for nine weeks with the use of progesterone implants. This was done, and 75 mgs. of progesterone were implanted subcutaneously on the tenth day after mating. The implants were removed two days before whelping was due and on the following day the bitch produced six normal healthy pups which she reared.

This case is most unusual as the bitch has probably twice re-absorbed the foetuses and yet was able to produce a normal litter /

litter afterwards. The cause of the re-absorption is due to the failure of the bitch to produce enough progesterone for the continuation of a normal pregnancy.

In this type of case the white cell counts are considerably above those seen in normal pregnancies but would be indistinguishable from the counts obtained from cases of pyometra.

CHANGES SEEN IN THE BLOOD PICTURE DURING FALSE PREGNANCY

Having described the changes in the blood picture associated with pregnancy, it was considered advisable to see what changes occurred in false pregnancy, to ensure that the two conditions could not be confused.

REVIEW OF THE LITERATURE

Only one direct reference to the blood picture in false pregnancy has been found and that is by Khuen, Park and Adler (1940) who give the details of two cases of false pregnancy. The article dealt mainly with pyometra. Their results in false pregnancy were as follows:

Age	R.B.C. millions per cu.m.m.	W.B.C. per cu.m.m.	Stabs. %	Juveniles %	Lymphs. %
7 years	5.22	10,000	5	0	27
3 years	6.54	10,500	8	0	28

No clinical pictures are given for these cases.

PRESENT WORK

Blood samples were removed from bitches showing clinical signs of pseudocyesis, and each sample was correlated with these signs. In some cases only one sample was taken, and in others serial samples were obtained. A total of eight bitches were used and twenty-three samples obtained. In the case of bitch No. P.6 samples were taken during oestrus and after oestrus until a false pregnancy developed.

RESULTS /

RESULTS

The blood pictures are given in the Appendix page 55.

The clinical history for each bitch will be given here.

Code No.	Age	Time after Oestrus	Clinical Signs
P.I.	2 years	Unknown	Swollen mammary glands and much milk present. Behaviour normal.
		Four days later	" " "
		Nine days later	Not so much swelling of glands but milk still present
P.6	1 year	Eight weeks	Glands swollen, milk in large quantities. Behaviour abnormal
		Nine weeks	Less milk
P.6	1½ years	Been in oestrus one week	Normal
		In oestrus two weeks	Normal
		In oestrus three weeks	Normal
		Finished oestrus four days	Glands swollen, No milk
		Twelve days	" "
		Three weeks	" "
		Four weeks	" "
		Five weeks	" "
		Eight weeks	Glands swollen. Much milk present. Behaviour abnormal
		Nine weeks	" " "

Code No.	Age	Time after Oestrus	Clinical Signs
P.17	7 years	Twelve weeks	Glands swollen, much milk present. Behaviour abnormal.
		Thirteen weeks	" " "
		Fourteen weeks	Less milk
P.81	4 years	Twelve weeks	Mammary glands very big. Lot of milk. Behaviour abnormal.
PHI.6	7 years	Twelve weeks	Lot of milk. Behaviour normal. Also has mammary tumours
PHI.11	3¾ years	Seven weeks	Little milk and slight glandular enlargement. Behaviour normal.
PHI.21	6 years	Seven weeks	Copious milk. Glands large. Behaviour normal.
PHI.32	10 years	Seven weeks	Behaviour very abnormal. Milk present

DISCUSSION

The results shown that there are no changes seen in the blood picture during false pregnancy, and the picture is unlike that seen in true pregnancy.

The red cell counts were normal and the sedimentation rates were only raised in one case. This bitch also had mammary tumours and the increased rate is probably not due to the false pregnancy.

The total white cell counts were low, the highest count being 12,125 per cu.m.m. and the lowest 6675 per cu.m.m. (average 9661 per cu.m.m.).

Platelet counts tended to be slightly raised and the average count was 294,000 per cu.m.m.

There did not appear to be any correlation between the blood picture and the clinical signs being shown. Bitches with normal behaviour and only a little milk present had blood pictures which were indistinguishable from those of bitches whose behaviour was abnormal and who had considerable hypertrophy of the mammaryglands.

In the case of P.6 from whom serial samples were taken, no specific changes in the blood picture were seen between the end of oestrus and the commencement of the clinical signs of false pregnancy.

EFFECTS OF EXERCISE ON NORMAL DOGS

REVIEW OF LITERATURE

Wells and Sutton (1915) observed that muscular exercise raises the red cell counts and give figures of a rise from 6.5 million per cu.m.m. to 7.1 million percu.m.m.

Wiggers (1949) statėd that erythrocyte counts are almost invariably increased after short periods of vigorous exercise, and he says that contraction of the spleen plays a dominant role in dogs in this respect. No figures are given.

Houssay (1955) says that changes in the blood depend on the intensity and duration of the exercise. Plasma volume diminishes without significant variation in red cell volume. The viscosity, specific gravity, and red cell count increase due to loss of fluid from the blood mainly to the tissues and the entrance of stored erythrocytes into the blood stream. No actual figures are given to show the extent of the variations.

Wittke (1960) found that the packed cell volumes rose in horses one minute after exercise started and remained at a new level after a further minute. The corpuscular volume appears to double and the rise is greatest if the horse is galloping rather than trotting.

PRESENT WORK

This work was undertaken to find out whether exercise causes variations in the blood picture which may be confused with pathological changes.

A /

A total of four dogs were used for this work and five sets of results were obtained. The kennel number of the dogs involved is given in the results, Appendix page 56. All the dogs were housed at the time in the College kennels and exercising took place on open grass land. Details of the amount of exercise are given in the results. No food was given for the twelve hours prior to exercise. The dogs were re-sampled as soon after exercise as possible, usually within five minutes.

Particulars of dogs used

Relevant results are given in the Appendix page 56.

R.D.V.C.2. Control For H.33 and P.77

Housed in kennels during the whole period and samples taken at exactly the same time as from the two dogs which were exercised. The weather was cold and snow was on the ground. Last feed twelve hours previously.

H.33

Exercised mainly at a brisk walk over a distance of two miles. After exercise respiration and heart rate accelerated but dog not exhausted. Weather cold and snowy.

P.77 (18 months)

Exercised herself very vigourously, running free over a distance of approximately four miles. After exercise respirations and heart rate much accelerated and she was tired. Weather cold and snowy.

R.D.V.C.4

Exercised for thirty minutes over a distance of three miles. Afterwards respirations and heart rate accelerated. Weather cold with snow.

P.44

Exercised free for twenty minutes. After exercise dog was not very distressed and respirations and heart rate were only a little above normal. Weather very cold with snow.

R.D.V.C.3 Control for R.D.V.C. 4 and P.44

Housed in kennels all the time. Weather cold with snow.

P.77 'b' (20 months old)

Exercised for 30 minutes at 10.30 a.m. for a further 35 minutes at 11.30 a.m. and two additional periods of 30 minutes at 1.30 p.m. and 3 p.m. The dog received no water at all from 8 a.m. until the periods of exercise were completed. The dog exercised herself freely on open land during each period and was physically tired at the end of the day. The weather was fine and warm the whole time. The total distance covered was approximately 12 miles.

RESULTS

The relevant results are given in the Appendix pages 56.

The following table shows the mean average results (excluding P.77'b') which were obtained.

		Before Exercise	After Exercise
Control	R.B.C. millions per cu.m.m.	6.64	6.61
2 dogs.	Specific Gravity	1.0627	1.0627
	W.B.C. per cu.m.m.	10,925	10,587
Exercise	R.B.C. millions per cu.m.m.	6.406	6.506
4 dogs	Specific Gravity	1.0613	1.0633
	W.B.C. per cu.m.m.	10,637	10,690

The results obtained from dog P.77'b' which was exercised and also deprived of water are in the Appendix page 56.

DISCUSSION OF RESULTS

The results obtained were similar to the results of the authors whose work has been reviewed. None of the results, however, indicate that the changes occurring in the blood after exercise can be confused with, or mistaken for, pathological changes.

The red cell counts rose slightly after exercise as did the haemoglobin and packed cell volumes. In no case were the figures outside the normal range. The specific gravities rose, indicating that fluid had been lost from the circulation to the tissues. The rise in total white cells is insignificant.

It must be remembered that although the exercise given to the dogs was vigorous and lasted twenty minutes or more, it was carried out in the main during cold weather. In very warm weather the rise in red cell counts and specific gravities would probably be greater, but owing to several factors it was not possible to carry most of these experiments out during really hot weather. Only in the case pf P.77'b' was the exercise undertaken during warm weather and in this case the red cell count rose by only 15% (700,000 per cu.m.m.) and the specific gravity from 1.0495 to 1.0525, even after the dog had been without water for seven and a half hours.

There were no changes seen in the differential white cell picture, platelet counts or sedimentation rates which could be correlated with the effects of exercise.

Only after very prolonged exercise undertaken during very hot /

hot weather would the rise in red cells and specific gravity be confused with any pathological condition. This state of affairs is unlikely to occur in a dog which may require examination for a suspected pathological abnormality.

CONCLUSIONS

The rise in red cell counts, haemoglobin, packed cell volumes and specific gravities occurring after exercise is not sufficient to be confused with any pathological abnormality. No other changes occurred in the blood picture as a result of exercise.

THE EFFECT OF FEEDING ON THE BLOOD PICTURE

This work was performed to try and clarify the information already available on this subject.

REVIEW OF THE LITERATURE

Pohl (1889) discussed the effects on the blood picture of feeding dogs on a variety of foods which included meat, water, fish, butter and starch. He obtained no rise in total white blood cell counts with some foods but obtained a rise in all of the twenty dogs used if meat was fed. The maximum elevation of the white counts occurred at intervals of one to six hours after food, the majority occurring one to two hours after food. The rise in white cells varied from dog to dog (on the same diet) from a rise of 35% up to 146%. (5,929 per cu.m.m. to 14,598 per cu.m.m. after one hour and 19,425 per cu.m.m. to 26,241 per cu.m.m. after two hours). Of ten dogs whose white cell counts were normal (below 14000 per cu.m.m.) at the start of the experiments, nine had white counts above the normal range after feeding meat.

Goodall, Lovell Gulland and Noel Paton (1903) obtained results indicating that there was a definite leucocytosis after feeding. The highest white cell counts occurred about four hours after the meal, and removal of the spleen did not affect the results. Their results can be summarised as follows:

The dog's total white cell counts rose to a maximum and then fell. Both neutrophils and lymphocytes were increased.

Dog /

Dog I. White blood cells rose from 15800 to 32200 per cu.m.m. Maximum values occurred four hours after food.

Dog II. White blood cells rose from 168oo to 45000 per cu.m.m. Maximum values occurred three hours after food.

Dog III. White blood cells failed to rise.

Two splenectomised puppies had white counts which rose from 11200 to 28200 per cu.m.m. three hours after food, and 18000 to 29600 per cu.m.m. five and a half hours after food respectively.

Nicholas and Cot (1905) used four dogs and took numerous samples following the feeding of a variety of foods. In Dog A a rise in white cells of over 500% occurred after meat was fed, but the rise was not quite so marked after carbohydrate foods. Only in the experiment where meat was given did the total white count rise above normal (3,600 per cu.m.m. to 19,100 per cu.m.m. two hours after meat).

In Dog B a rise always occurred after food, especially meat, but again only once did the total white count exceed the normal value (7810 per cu.m.m. rose to 21,200 per cu.m.m. four hours after food). The maximum rise was over 250%.

Out of eight experiments on Dog C the total white counts rose in four cases and fell in four, and out of seven experiments on Dog D (splenectomised) the white count rose in five cases and fell in two after feeding. The maximum rise was after beef and water (7500 per cu.m.m. to 17400 per cu.m.m. four hours after food).

The /

The authors considered that a rise in white cells appears to follow feeding. More often than not, however, this rise never elevated the total white count above the normal limits when food other than beef was fed. Feeding beef caused a large increase in white blood cells. The amount of food eaten did not affect the extent of the rise in white cells and the increase occurred to a greater extent in some dogs than in others. Splenectomy did not affect the results. They also noted that a rise will occur at the normal feeding time even if no food was actually given.

Scarborough (1931) quotes Schultz (no reference given) as saying that there is a rise in the white cell count of 100 to 150% forty-five minutes after feeding, and that this rise lasts for several hours.

Blakemore (1964) states that leucocytosis occurs in young dogs following feeding.

PRESENT WORK

Owing to the differences in the results obtained by earlier workers it was considered desirable to repeat the work to see if large increases in total white cell counts occurred. This was done mainly to ascertain if the rise in white cells could be confused with any pathological increase.

Four dogs were used in groups of two. A control animal was used with each group of two dogs.

All dogs were housed in kennels during the experiments.

Two /

Two dogs were fed, after an initial blood sample had been taken, with ½ lb. of cooked meat and ¼ lb. of biscuit meal. The control animal was not fed. Blood samples were taken at varying intervals after feeding. None of the animals had been fed for twenty-four hours prior to the experiment commencing.

RESULTS

The results are given in the Appendix pages 57 and 58 together with the results from the Controls.

DISCUSSION OF RESULTS

The results obtained indicated that the total white cell count rose in three out of the four dogs which received food. This rise appeared to reach a maximum one or two hours after feeding and then the number of white cells fell, returning to normal some four hours after food was ingested. The variations between some of the results are no more than the hourly variations seen in normal dogs. In one dog no rise occurred and the white cell count in fact fell slightly. One animal used as a control showed no alteration while the second control showed a slight rise in total white cells.

The maximum percentage alteration in total white cells when compared with the first sample is as follows:

Control S.23 = - 11% (fourth sample)
~~Control~~ S. 3 = - 24% (four hours after food)
~~Control~~ S. 4 = + 23% (two hours after food)
Control R.D.V.C.2 = + 9% (third sample)
~~Control~~ R.D.V.C.1 = + 35% (one hour after food)
~~Control~~ P.77 = + 26% (one hour after food)

The rise in white cells was due in one case to an increase in neutrophils, lymphocytes and monocytes and in the other two cases to neutrophils and monocytes alone. No shift to the left was observed.

The specific gravities in all four experimental dogs rose while the controls showed no alteration. In three cases the maximum rise was obtained one to two hours after food and in the fourth case three and a half hours after food.

The packed cell volumes increased slightly in all four cases, and showed a maximum rise at the same time as the specific gravities.

Haemoglobin values rose in three cases and fell in one case. It is possible that this apparent rise was not entirely due to an increase in haemoglobin, but also due to an increase in the lipid content of the plasma which interfered with the colorimetric readings.

The platelet counts rose in all four experimental dogs one to two hours after food and then fell again. Platelet counts were not carried out in all instances and therefore the true picture of this increase was not revealed. Both controls showed a slight increase so it is probable that the rise was due in part to the repeated blood sampling rather than to the feeding.

The results indicate that the total white cell count rises usually one to two hours after feeding due to an increase in neutrophils, monocytes and lymphocytes. This rise does not occur in /

in all dogs, and in this respect the results agree with those of Goodall *et al.* and Nicholas and Cot. I was unable to obtain the very large increases seen by the authors reviewed and could not confirm their results. If raw meat had been fed a greater rise might have occurred but the dogs were kept on their normal diet for this work. As most of the pet dogs and all kennelled dogs used in other parts of this work were normally fed cooked meat this experiment was confined to the use of cooked meat.

The haemoglobin, packed cell volumes and specific gravities also tended to rise after feeding but again the rise was only slight and often no more than would occur in normal hourly variations.

Although the number of dogs used was small, it is considered that the rise in total white cells after feeding is not sufficient to be confused with a pathological increase. Only in dogs whose normal white count lies at the top of the normal range would any confusion with pathological leucocytosis occur.

As the results obtained did not agree with those of other authors in that the total white cell counts did not show any spectacular rise after feeding, further work is required on this subject. Experiments using a larger number of young dogs and a variety of foods are needed to confirm or refute the work done sixty or more years ago. Unfortunately experimental material was not available for this to be done during the present study.

THE EFFECT OF TRAVELLING ON A DOG'S BLOOD PICTURE

This section of the work was undertaken to ascertain whether or not travelling by car altered the blood picture of dogs at all. As many cases treated in Veterinary Hospitals arrive by car it was considered desirable to find out if the blood picture of such dogs was altered enough for the changes to be confused with pathological changes.

REVIEW OF THE LITERATURE

No trace has been found of any work having been done on this particular aspect of the canine blood picture.

PRESENT WORK

Four dogs and two controls were used in the work. The dogs were sampled prior to being taken out in a car and sampled again immediately after the journey was completed. The controls were housed in Kennels all the time and sampled at the same time as the other dogs. The four dogs used had never been in a car prior to this work.

The main results are given in the Appendix page 59.

DISCUSSION OF RESULTS

The results obtained indicate that there is no marked change in a dog's blood picture after travelling, even if the dog is not used to a car. None of the dogs used was actually sick and it proved impossible to obtain such a dog for this work. A dog which was continuously sick while travelling may have an altered blood /

blood picture and it would be advisable to leave this dog to settle down before blood sampling was attempted.

No significant alteration in the blood picture occurred consistently in any of the four dogs except for a fall in eosinophils.

	Mean average Eosinophils before experiment	Mean average Eosinophils after experiment
Controls	558 per cu.m.m.	528 per cu.m.m.
Travelling Dogs	777 per cu.m.m.	368 per cu.m.m.

The average decrease in absolute numbers of eosinophils was a little over 50%. This is due to fear rather than to the direct effects of travelling, and in a dog which is terrified of travelling the eosinophil count may fall to nothing.

It is well known that the eosinophils will fall markedly provided that adrenal cortical function is normal, when A.C.T.H. is administered to dogs or humans; Martin, Skillen and Deubler (1954); Cape, Thomas and Palmer (1952); Ogryzlo and Gornall (1952); Best, Muehrcke and Kark (1952); Hernández-Morales, Casas and Garcia-Sanz (1950); and Thorn, Forsham and Garnet Prunty and Hills (1948).

The administration of A.C.T.H. is really an artificially produced fear reaction and the more frightened the dog is while travelling the lower will be its eosinophil count.

If a dog has a pathologically elevated eosinophil count, as would occur in certain skin diseases, then travelling may reduce the count if the dog is not used to travelling, and give an erroneous differential and total white cell picture. In such instances it would be wise to delay blood sampling for twenty-four hours, so that the blood picture may return to its former state.

THE EFFECT OF LACK OF WATER ON THE NORMAL DOG'S BLOOD PICTURE

Although there has been much written about the effects of dehydration on the blood picture in pathological cases, little work appears to have been done on the effects of lack of water, for periods of up to twenty-four hours, in normal dogs. This fact appears important as some dogs presented for veterinary examination have come long distances by road and have had no water on the way. This work is designed to find out if such water lack can be confused with true pathological dehydration. The effects of long term water lack were also studied. No previous work appears to have been done on this aspect.

REVIEW OF THE LITERATURE

Underhill and Kapsinow (1922) used five female dogs kept in metabolism cages, without food or water, for periods of several days. Only haemoglobin values were calculated. During the first twenty-four hours three dogs showed the following increases in haemoglobin values, 10%, 13% and 14%. A fourth dog had a rise of 22% after eighteen hours but this fell to 12% above normal after twenty-four hours. After three days marked changes occurred due to haemo-concentration.

De Boer (1945) investigated the effects of chronic dehydration by leaving dogs without food or water for periods of seven to ten days. He obtained haemoconcentration of the blood, with increases in haemoglobin, specific gravity and red cell counts.

Controls /

Controls were used. On the first and second days the values fluctuated. The haemoglobin increased 10% after twenty-four hours but was normal on the second day. Total red cell counts rose by 20% after twenty-four hours but were below the initial value on the second day. The specific gravity rose by 5% on the first and second days. Not until the third or fourth day were really marked changes observed. Haemoconcentration occurred but was kept within limits by the withdrawal of fluid from the body cells and a subsequent less of weight.

Schalm (1961) gives a good example of the effects of dehydration. The figures given are from a dog which was markedly dehydrated on arrival in hospital but whose water balance returned to normal after ten days.

	Arrival	First Day	Fourth Day	Tenth Day
Hb. grms/100 mls.	13.6	9.0	9.8	9.0
R.B.C. millions/cu.m.m.	7.25	5.5	6.14	4.83
P.C.V. %	43	31	32.5	30

PRESENT WORK ON ACUTE WATER DEPRIVATION

The previous work was done on dogs kept in cages. It was considered desirable to find out if the same results would be obtained from dogs which were deprived of food and water for periods not exceeding twenty-four hours, but which led an otherwise normal life during the period. All the dogs used were exercised as normal during the period of water deprivation.

Two /

Two dogs were used with one control, which was allowed water *ad lib.* Another dog was studied without a control. The main results are given in the Appendix page 60.

DISCUSSION OF RESULTS

The results obtained are similar to those in the literature on the subject. One dog showed an increase in haemoglobin, packed cell volume, red cell count and specific gravity after water deprivation but the control dog also showed a similar increase. The other dog deprived of water showed no significant change.

The conclusions which can be drawn from this work are the same as those of other workers although the number of dogs used was admittedly small. The red cell counts, haemoglobin, packed cell volume and specific gravity may be increased by depriving an animal of water for periods of twenty-four hours, but this increase should not be confused with pathological dehydration. The changes seen are no more than in the normal daily variations of a dog's blood picture.

Further work could be done to find out if the changes are more marked in dogs deprived of water during very hot weather. It was not possible to do this during the present study.

THE EFFECTS OF LONG TERM WATER DEPRIVATION ON DOGS

The work done by other authors is concerned with the effects on the blood picture of a complete lack of water over a long period. This section is concerned with the effects of a long term shortage of water. Such a shortage of water is due either to the dogs being given access to water only once or twice a day, to irregularities in the system of watering, or to the dogs spilling the water they are given and the water not being replaced.

Such conditions occur especially in kennels where a large number of dogs are housed and either irregularities in routine, or lack of staff, allow water shortage to occur. These conditions occurred in one of the kennels from which dogs were obtained. This was Kennel I. Two visits were made to this kennel to bleed beagles, and there was a gap of nine months between the two visits. The type of dog, housing and feeding did not alter between these two visits but the system of watering did. When the kennels were first visited there was adequate kennel staff, and watering took place several times daily, but on the second visit the kennels were short staffed. This meant that the dogs did not get fresh water as often as /

as before. Water was given after feeding each day but as the dogs were housed in groups of ten or more the water trough often got knocked over. If this happened no more water was available until the next day.

The dogs appeared to be used to this lack of water and had adapted themselves to taking water infrequently. Even if water was available they often refused it and were able to live quite normally with very little water per day - much less than the normal daily intake of a household pet. This phenomenon has also been seen in individual dogs living in conditions where water was given infrequently.

The differences seen between samples taken at the first visit and those taken at the second visit is marked, and the differences are due to this water shortage.

RESULTS

The results are given in two groups. Firstly, the results from dogs sampled on the first visit when plenty of water was available, and secondly, results from dogs of the same age sampled on the second visit when watering was infrequent. The full results are given in the Appendix page 61.

DISCUSSION OF RESULTS

The two sets of results described show a marked difference. The red cell counts, packed cell volumes, haemoglobin and specific gravities are higher in the group getting water infrequently than in the group receiving regular water supplies. The white cell counts vary considerably from dog to dog but no specific difference was seen between one group and the other. These differences are not statistically significant but are of definite clinical significance, especially with regard to the diagnosis of anaemic conditions.

The chronic shortage of water has no visible effect on the animal's health but causes sub-clinical dehydration. This has the effect of appearing to raise the mean red cell counts by 750,000 to 1,000,000 per cu.m.m., the packed cell volumes by 5 to 6%, the haemoglobin by 2 to 2.3 grms. per 100 mls. and the specific gravities by 1.0057 to 1.0085. This rise is of course due to a drop in plasma volume and not to an increase in the cellular content of the blood. The difference is seen most markedly in the dogs aged less than one year. In the case of adults they are housed together in smaller numbers and get more individual attention and the chances of the water bowls being knocked over is less.

CONCLUSIONS

Water deprivation for a period of up to twenty-four hours has no marked effect on the blood picture.

Dogs living in conditions where watering is infrequent show /

show raised red cell counts, packed cell volumes, haemoglobins and specific gravities. The effects are most marked in young dogs housed together in large numbers, as the water is often spilled only shortly after being given.

RED CELL FRAGILITY

This particular estimation was carried out on only a few of the dogs examined, and not as a routine examination. A total of twenty-five samples from twenty-five dogs were examined by the method described in the section dealing with materials and methods. These dogs came from all the sources already described and were of mixed breeds and ages.

RESULTS AND DISCUSSION

The results show the following:-

The average solution in which initial haemolysis occurred was 0.596% saline.

Range of variation was 0.650% to 0.525%.

The average solution in which haemolysis was completed was 0.389% saline.

Range of variation was 0.425% to 0.350%.

There did not appear to be any variations due to breed or to environment. Dogs from the same environment did, however, show considerable variations one from the other. In the case of H.29 this bitch was eight weeks pregnant and did not show any signs of increased fragility.

These results vary from those of Musser and Krumbhaar (1916) whose average results were 0.462 to 0.330% for initial and complete haemolysis. Albritton (1955) gives initial haemolysis as 0.45% and complete haemolysis as 0.36%.

CLOTTING TIMES FOR NORMAL DOGS

This section, like that on red cell fragility, was done on only a few dogs. Twenty-seven dogs were examined using the method already described. Dogs of all ages and breeds were used.

RESULTS

The results are given in the Appendix page 62.

Each set of results is divided into two parts. The first reading shows the time taken for clotting to commence, e.g. lumps appeared in the tube when it was inverted. The second reading is the time taken for a solid clot to form and this clot stuck to the bottom of the tube when it was inverted.

The average time taken for the clotting to start was three minutes twenty seconds, and the range of variation one minute thirty seconds to seven minutes.

The average time taken for the clot to be complete was four minutes fifty seconds, and the range of variation was one minute forty-five seconds to nine minutes thirty seconds.

In three cases an initial clot was formed but a complete clot which would stick to the tube was not formed. This failure to clot completely is possibly due to spontaneous fibrinolysis. In humans it is known that any form of stress can produce fibrinolytic activity in the blood. (Biggs and Macfarlane 1957).

Three of the dogs used were in oestrus and this had no apparent effect on the clotting times. The clotting time was also normal in a bitch which had whelped three weeks previously.

These results are the same as those of Watt (1964) using the same techniques.

STANDARD BLOOD PICTURE FOR NORMAL HEALTHY DOGS

The work so far described in this thesis makes it quite clear that it is impossible to derive a standard blood picture for normal dogs without introducing several modifying factors. It has already been shown that age, environment, pregnancy, chronic water deprivation, and in the case of greyhounds, breed, all play an important part in regulating the blood picture. The other factors discussed do not influence the blood picture appreciably and can be discounted for the purposes of this section.

Pregnancy is a recognisable clinical entity and so it need not be introduced as a modifying factor when deriving a standard blood picture for normal dogs. The other factors mentioned, however, have to be introduced and so for this reason the dogs have been grouped according to environment and age in order to establish the standard blood picture for the dogs used in this work. In the case of greyhounds they have been classed also according to breed.

Pregnant bitches, and bitches in oestrus, are excluded from the results.

RESULTS

Dogs from all sources have been used and have been classed into the following groups:

1) Greyhounds from both Kennel C and the pets group.
2) Dogs from large kennels (housing one hundred dogs or more) Kennel I.
3) Dogs from small kennels (housing less than one hundred dogs).

   In this group dogs from Kennels S, H. and R.D.V.C. have been grouped together. In these kennels the number of dogs housed was actually less than fifty.

4) /

4) Pet dogs - both town and country pets, together with working dogs, have been put in this group. Some of the working dogs were kennelled but never in numbers of more than six together, and so have been included here.

Each group of dogs was sub-divided into the following age groups:- one day old, one to three months, three to six months, six months to one year and one year and over. The number of dogs used to compile the standard blood picture are as follows:-

	Greyhounds	Large Kennels	Small Kennels	Pet Dogs
1 day old	-	-	-	4 samples 4 dogs
1-3 months	12 samples 6 dogs	-	6 samples 3 dogs	-
3-6 months	17 samples 6 dogs	16 samples 16 dogs	32 samples 18 dogs	-
6 months to 1 year	27 samples 14 dogs	6 samples 6 dogs	27 samples 21 dogs	12 samples 11 dogs
1 year and over	58 samples 46 dogs	25 samples 25 dogs	40 samples 28 dogs	106 samples 103 dogs

The average results together with the standard deviation was worked out for each group of dogs. Some of the groups give results which are very similar to each other and this is shown in the Tables. The results for day old puppies will be considered separately. If the standard deviation is added and also subtracted from the average figure, this will give a range of variation into which approximately 75% of the normal dogs in each group will fall. If twice the standard deviation is added or subtracted /

subtracted from the average figure, the resulting range of variation will cover well over 90% of the normal values in any group.

<u>Red cell counts, Packed cell volumes, Haemoglobin and Specific Gravity</u>

The results for each group are given in the following tables.

RED CELL COUNTS, PACKED CELL VOLUMES

	Age		Greyhounds	Large Kennels	Small Kennels	Pets
R.B.C. millions per cu.m.m.	1-3 months	Average	4.420	-	5.061	-
		Standard Deviation	± 0.676	-	± 0.268	-
	3-6 months	Average	6.050	5.426	5.418	-
		Standard Deviation	± 0.545	±0.770	± 0.474	-
	6 months to 1 year	Average	7.054	6.481	6.396	6.038
		Standard Deviation	± 0.473	± 0.237	± 0.544	± 0.434
	1 year and over	Average	7.463	6.981	6.577	6.440
		Standard Deviation	± 0.428	± 0.493	± 0.531	± 0.729
P.C.V. %	1-3 months	Average	34.1	-	37.8	-
		Standard Deviation	± 5.8	-	±3.2	-
	3-6 months	Average	47.0	39.2	41.4	-
		Standard Deviation	± 3.7	± 5.8	± 3.2	-
	6 months to 1 year	Average	53.5	48.1	47.3	47.2
		Standard Deviation	± 3.2	± 1.4	± 3.9	± 2.9
	1 year and over	Average	59.1	52.1	50.1	48.7
		Standard Deviation	± 3.3	± 2.4	± 2.8	± 5.0

The /

The table shows that greyhounds over three months of age have appreciably higher red cell counts and packed cell volumes than dogs of the same age from other sources. The results for dogs living in large kennels are conditioned to some extent by water deprivation and would be lower if water had been available at all times. There is little variation between the values obtained from pet dogs and those from dogs living in large and small kennels.

Standard Blood Picture

HAEMOGLOBIN AND SPECIFIC GRAVITIES

	Age		Greyhounds	Large Kennels	Small Kennels	Pets
Hb. grms/100 mls.	1-3 months	Average	11.1	-	13.0	-
		Standard Deviation	± 2.4	-	± 0.9	-
	3-6 months	Average	16.1	13.0	14.5	-
		Standard Deviation	± 1.5	± 2.3	± 1.4	-
	6 months to 1 year	Average	18.6	16.6	16.8	16.6
		Standard Deviation	± 0.97	± 0.5	± 1.6	± 1.0
	1 year and over	Average	20.8	18.1	17.9	17.8
		Standard Deviation	± 1.1	± 1.1	± 1.1	± 1.5
Specific Gravity	1-3 months	Average	1.0443	-	1.0501	-
		Standard Deviation	± 0.0038	-	± 0.0028	-
	3-6 months	Average	1.0566	1.0519	1.0533	-
		Standard Deviation	± 0.0042	± 0.0043	± 0.0034	-
	6 months to 1 year	Average	1.0638	1.0644	1.0602	1.0603
		Standard Deviation	± 0.0020	± 0.0022	± 0.0052	± 0.0033
	1 year and over	Average	1.0679	1.0675	1.0639	1.0617
		Standard Deviation	± 0.0017	± 0.0016	± 0.0027	± 0.0045

These results show the same pattern as the ones for red cell counts and packed cell volumes. Greyhound values are high while the results from pet dogs and dogs from large and small kennels show a close similarity. The specific gravity values for dogs in large kennels are appreciably raised due to water deprivation. The larger standard deviations seen in adult pet dogs is to be expected as they came from very varied environments.

CORPUSCULAR VALUES

The average results from the four groups are given in the following table. Day old puppies are considered separately.

Average Values and Standard Deviations

	Age		Greyhounds	Large Kennels	Small Kennels	Pets
M.C.V. cu.μ.	1-3 months	Average	77.0	-	74.6	-
		Standard Deviation	± 3.3	-	± 2.9	-
	3-6 months	Average	77.7	72.3	76.8	-
		Standard Deviation	± 2.5	± 3.0	± 5.3	-
	6 months to 1 year	Average	76.1	74.6	73.8	78.1
		Standard Deviation	± 3.3	± 1.8	± 3.4	± 5.5
	1 year and over	Average	79.3	74.2	76.2	77.4
		Standard Deviation	± 3.2	± 3.6	± 4.9	± 4.0
M.C.H. μμ. grms.	1-3 months	Average	25.0	-	25.7	-
		Standard Deviation	± 2.1	± -	± 1.2	-
	3-6 months	Average	26.6	23.8	27.0	-
		Standard Deviation	± 0.9	± 1.4	± 2.2	-
	6 months to 1 year	Average	26.5	25.6	26.2	27.6
		Standard Deviation	± 1.2	± 0.8	± 1.9	± 1.1
	1 year and over	Average	27.9	25.7	27.3	27.8
		Standard Deviation	± 1.4	± 1.4	± 1.7	± 2.0
M.C.H.C. %	1-3 months	Average	32.4	-	34.5	-
		Standard Deviation	± 1.9	± -	± 2.5	-
	3-6 months	Average	34.3	33.0	35.0	-
		Standard Deviation	± 1.0	± 2.2	± 1.9	-
	6 months to 1 year	Average	34.7	34.0	35.6	35.1
		Standard Deviation	± 0.3	± 0.5	± 1.3	± 1.3
	1 year and over	Average	35.0	34.5	35.8	35.8
		Standard Deviation	± 1.0	± 0.9	± 1.4	± 1.1

No very obvious differences are seen between the values from each group. Greyhounds tend to have slightly higher M.C.V. values but the difference is very small. The values for greyhounds should still, however, be classed separately from those of other breeds

The M.C.H.C. values obtained appear rather high when compared with the results from other authors. Schalm (1961) states that a M.C.H.C. value greater than 35% is of doubtful value unless a micro-haematocrit has been used for the packed cell volume estimations.

It must be emphasised that the corpuscular values presented here are those obtained by the methods used in this work. If a different method of obtaining P.C.V.s or haemoglobin had been employed the values would have been lower.

RETICULOCYTES AND NORMOBLASTS

The average results are given in the table which follows. Apart from the fact that the values for kennel dogs tended to remain high for a longer period, e.g. up to six months of age, there are no real differences between the results from each group.

Average Values and Standard Deviations

	Age		Greyhounds	Large Kennels	Small Kennels	Pets
Reticulocytes %	1-3 months	Average	3.38	-	1.88	-
		Standard Deviation	± 2.86	-	± 0.43	-
	3-6 months	Average	0.20	1.05	1.43	-
		Standard Deviation	± 0.26	± 0.50	± 2.03	-
	6 months to 1 year	Average	-	0.53	0.45	0.21
		Standard Deviation	± -	± 0.31	± 0.68	± 0.15
	1 year and over	Average	0.04	0.30	0.66	0.18
		Standard Deviation	± 0.06	± 0.20	± 0.67	± 0.21
Normoblasts per cu.m.m.	1-3 months	Average	155	-	146	-
		Range	0 - 790	-	20 - 380	-
	3-6 months	Average	6	50	86	-
		Range	0 - 72	0 - 157	0 - 1200	-
	6 months to 1 year	Average	Nil	28	36	3
		Range	Nil	0 - 126	0 - 300	0-24
	1 year and over	Average	Nil	14	28	2
		Range	Nil	0 - 112	0 - 450	0-37

SEDIMENTATION RATES

The average results are given in the next table. No real differences are seen between the groups except that the rates for young dogs in large kennels were higher than those for the other groups.

SEDIMENTATION RATES m.m. per hour

Average Values and Standard Deviations

Age		Greyhounds	Large Kennels	Small Kennels	Pets
1-3 months	Average	1.68	-	2.8	-
	Standard Deviation	± 1.88	-	± 0.35	-
3-6 months	Average	0.11	6.3	1.11	-
	Standard Deviation	± 0.22	± 5.0	± 1.34	-
6 months to 1 year	Average	Nil	0.2	0.52	0.4
	Standard Deviation	± -	± 0.7	± 0.54	± 0.48
1 year and over	Average	Nil	0.3	0.17	0.2
	Standard Deviation	± -	± 0.8	± 0.64	± 0.7

PLATELET COUNTS

The results are given in the following table. Greyhounds have slightly lower average platelet counts than the other groups and dogs in large kennels higher average results.

PLATELET COUNTS (thousands per cu.m.m.)

Average Values and Standard Deviations

Age		Greyhounds	Large Kennels	Small Kennels	Pets
1-3 months	Average	353	-	411	-
	Standard Deviation	± 78	-	± 59	-
3-6 months	Average	247	378	390	-
	Standard Deviation	± 53	± 76	± 111	-
6 months to 1 year	Average	220	376	291	288
	Standard Deviation	± 34	± 58	± 51	± 70
1 year and over	Average	218	337	271	259
	Standard Deviation	± 52	± 69	± 75	± 60

TOTAL WHITE CELL COUNTS

The average results are given in the following table.

TOTAL WHITE CELL COUNTS (per cu.m.m.)

Average Values and Standard Deviations

Age		Greyhounds	Large Kennels	Small Kennels	Pets
1-3 months	Average	12,688	-	15,415	-
	Standard Deviation	± 1,828	-	± 2,878	-
3-6 months	Average	9,327	17,962	14,177	-
	Standard Deviation	± 1,515	± 2,850	± 2,800	-
6 months to 1 year	Average	8,931	16,657	11,070	10,229
	Standard Deviation	± 1,223	± 2,744	± 2,055	± 2,680
1 year and over	Average	7,860	15,134	10,514	10,137
	Standard Deviation	± 1,504	± 3,070	± 2,466	± 2,484

Each group shows distinct differences, apart from the pet dogs and the dogs aged six months or more from small kennels, whose results are similar.

DIFFERENTIAL WHITE CELL COUNTS (per cu.m.m.)

Average Values and Standard Deviations

Age	Type of White Cell		Greyhounds	Small Kennels
1-3 months	Neutrophil	Average	6,402	8,148
		Standard Deviation	± 737	± 2,060
	Meta.	Average	110	329
		Standard Deviation	± 75	± 183
	Band	Average	1,150	1,406
		Standard Deviation	± 308	± 479
	Seg.	Average	5,141	6,412
		Standard Deviation	± 736	± 1,570
	Lymph.	Average	4,473	6,018
		Standard Deviation	± 881	± 1,219
	Mono.	Average	247	536
		Standard Deviation	± 81	± 217
	Eosin.	Average	1,440	464
		Standard Deviation	± 880	± 281
	Misc.	Average	124	248
		Standard Deviation	± 72	± 118

These values show that mature neutrophils and lymphocytes are higher in dogs from small kennels than from greyhounds. The higher eosinophil counts in greyhounds are due to ascarid infestation (not clinically apparent).

DIFFERENTIAL WHITE CELL COUNTS (per cu.m.m.)

Average Values and Standard Deviations

Age	Type of White Cell		Greyhounds	Large Kennels	Small Kennels
3-6 months	Neutrophil	Average	4,811	10,718	7,909
		Standard Deviation	± 852	± 2,226	± 1,722
	Meta.	Average	41	445	225
		Standard Deviation	50	251	137
	Band	Average	425	1,649	1,124
		Standard Deviation	± 225	± 874	± 576
	Seg.	Average	4,344	8,622	6,558
		Standard Deviation	± 791	± 2,050	± 1,808
	Lymph.	Average	3,199	5,314	5,037
		Standard Deviation	± 896	± 384	± 1,315
	Mono.	Average	292	854	497
		Standard Deviation	± 98	± 404	± 246
	Eosin.	Average	942	864	618
		Standard Deviation	± 624	± 463	± 526
	Baso.	Average	-	17	1
		Standard Deviation	-	± 62	± 4
	Misc.	Average	80	195	112
		Standard Deviation	± 76	± 248	± 157

The highest values of mature neutrophils are seen in dogs from large kennels and the lowest values in greyhounds. The differences are considerable. Both lymphocytes and monocytes are higher in the kennelled dogs than in greyhounds. Eosinophils are only slightly higher in greyhounds than in the other dogs.

DIFFERENTIAL WHITE CELL COUNTS (per cu.m.m.)

Average Values and Standard Deviations

Age	Type of White Cell		Greyhounds	Large Kennels	Small Kennels	Pets
6 months to 1 year	Neutrophil	Average	4,570	10,349	6,349	5,579
		Standard Deviation	± 570	± 1,928	± 1,482	± 1,240
	Meta.	Average	26	126	159	133
		Standard Deviation	± 28	± 160	± 129	± 78
	Band	Average	455	1,215	936	796
		Standard Deviation	± 215	± 156	± 565	± 469
	Seg.	Average	4,087	9,007	5,254	4,648
		Standard Deviation	± 558	± 1,983	± 1,201	± 1,119
	Lymph.	Average	3,181	4,735	3,607	3,477
		Standard Deviation	± 681	± 1,566	± 1,318	± 1,220
	Mono.	Average	347	836	565	474
		Standard Deviation	± 135	± 379	± 303	± 219
	Eosin.	Average	771	656	428	591
		Standard Deviation	± 497	± 232	± 263	± 400
	Misc.	Average	60	80	120	104
		Standard Deviation	± 61	± 169	± 124	± 80

Segmented neutrophils and lymphocytes are highest in dogs from large kennels, while greyhounds again show the lowest values. Results from small kennels are slightly higher than from pet dogs. Eosinophils show slight variations only, but again are highest in greyhounds, while monocytes are highest in dogs from large kennels.

DIFFERENTIAL WHITE CELL COUNTS (per cu.m.m.)

Average Values and Standard Deviations

Age	Type of White Cell		Greyhounds	Large Kennels	Small Kennels	Pets
1 year and Over	Neutrophil	Average	4,909	9,873	6,936	6,347
		Standard Deviation	± 1,405	± 2,610	± 2,070	± 1,904
	Meta	Average	32	206	211	69
		Standard Deviation	53	223	218	96
	Band	Average	447	1,073	1,161	633
		Standard Deviation	± 314	± 699	± 726	± 450
	Seg.	Average	4,430	8,593	5,563	5,644
		Standard Deviation	± 1,318	± 2,082	± 1,625	± 1,670
	Lymph.	Average	1,878	3,672	2,466	2,475
		Standard Deviation	± 748	± 1,003	± 930	± 1,177
	Mono.	Average	426	848	533	549
		Standard Deviation	± 186	± 262	± 306	± 268
	Eosin.	Average	608	609	509	716
		Standard Deviation	± 477	± 494	± 416	± 489
	Misc.	Average	37	114	68	47
		Standard Deviation	± 52	± 113	± 102	± 63
	Baso.	Average	2	17	-	-
		Standard Deviation	-	± 30	-	-

In this group all the values from the four sources are closer together than before. Segmented neutrophils are still highest from large kennels and lowest from greyhounds. Lymphocytes and monocytes are again greater in number in dogs from large kennels. Eosinophils show little variation.

STANDARD BLOOD PICTURE FOR DAY OLD PUPS

R.B.C. millions per cu.m.m.	P.C.V. %	Hb. gms/100 mls.	Sed. Rate m.m. per hr.	Platelets thousands per cu.m.m.	M.C.V. cu.µ.
3.662	34.7	12.3	1/6th.	257	95.1
3.450-4.010	31 - 37	12 - 12.7	Nil - ½	199 - 264	89.8-100.2

M.C.H. µµ. grms	M.C.H.C. %	W.B.C. per cu.m.m.	Neut. per cu.m.m.	Meta. per cu.m.m.	Band per cu.m.m.
33.8	35.5	7894	5742	262	823
31.7 - 34.9	33.9-38.7	7421 - 8691	5418-6344	163 - 381	508 - 1141

Seg. per cu.m.m.	Lymph. per cu.m.m.	Mono. per cu.m.m.	Eosin. per cu.m.m.	Baso. per cu.m.m.	Misc. per cu.m.m.
4656	1444	523	174	-	9
4389 - 4851	1299 - 1679	445 - 627	74 - 391	-	Nil - 37

Values for red cells, packed cell volumes, and haemoglobin are lower than in older dogs, and M.C.V. values are higher. Total white cell counts and lymphocyte counts are low in comparison with the values seen in adult dogs.

MORPHOLOGY AND CHARACTERISTICS OF BLOOD CELLS FROM NORMAL DOGS

As there are considerable variations in each particular type of blood cell, the cells were studied to identify variations which are seen in normal healthy dogs.

REVIEW OF THE LITERATURE

Sherrington (1894) described the features of various blood cells. His terms, however, are not those recognised to-day and it is often difficult to identify the cells described.

Busch and Van Bergen(1902) describe the cellular elements very well. They say that neutrophils with a nucleus which is not completely segmented is the commonest form.

Lymphocytes have only small amounts of cytoplasm. The nucleus may be double, pyknotic or mural. This latter is the description of a nucleus with a clear central area in it. They also mention that granules occasionally occur in the cytoplasm of lymphocytes. The eosinophil granules varied in size and were relatively few in number.

Kohanawa (1928) described the cells in the blood stream. He described both small and large lymphocytes and recognised the presence of azurophilic granules in six per cent of small lymphocytes and one per cent of large lymphocytes. He described large lymphocytes and also transitional forms. The commonest fofm of mature neutrophil had four or five lobes to the nucleus, while the commonest eosinophil had only three lobes.

Alexandrov /

Alexandrov (1930) described the appearance of Gumprecht shadow cells (0 - 2.25%), reticulo-endothelial cells (0 - 0.25%) and Türk cells (0.5 - 1.75%) in the blood of normal dogs.

Scarborough (1931) described normal red cells as "round, bi-concave discs, rather pale, often appearing as ring forms. There is considerable anisocytosis".

The white cells are described in more detail and the following main points are made. Polymorphonuclear neutrophils have a sharply defined nucleus appearing as a twisted coil or irregular spiral, sometimes S-shaped composed of areas of dark blue chromatin which are arranged without order. Granules are present and the protoplasm is weakly neutrophilic.

Lymphocytes have rounded, occasionally oval or, rarely, indented nuclei. The nucleus is sharply defined and pyknotic. There is a small ring of homogeneous cytoplasm which sometimes contains a few distinct azurophile granules.

Monocytes are large and have an eccentrically placed nucleus which is bean-shaped or rounded. The large amounts of cytoplasm are weakly basophilic.

The nucleus of the eosinophil is irregular in shape, well defined and occupies one-third of the cell. The granules are oval, rod-shaped or irregular, and vary in size, shape and number. These granules have a weak affinity for acid stains and stain poorly with Wright's stain.

Basophils /

Basophils have a vesicular nucleus occupying half to two-thirds of the cell. The granules are fine, abundant and basophilic. They vary in size and are usually round.

Bloom and Meyer (1944) stated that pathological lymphocytes may be seen in the blood of normal dogs. These have deeply basophilic cytoplasm and small vacuoles may occur in the nucleus or cytoplasm.

Schalm (1961) described the peripheral blood cells in detail. The main facts not already described in this review are as follows:-

Rouleux formation is common and occasional "target cell" erythrocytes are seen in normal blood.

Platelets show extreme variation in size, shape, colour and arrangement. Usually they are small with a central cluster of azurophilic granules surrounded by a pale blue matrix. Occasionally long thread-like processes project outwards. Giant forms may be present.

Neutrophilic nuclei have lobes, the majority of which are separated by narrowing of the nucleus and not filament formation. The female nuclear sex bud is described. Toxic granulation is extremely rare in the dog, and toxic changes cause vacuolation and fenestration of the cytoplasm.

The band neutrophil is described as having a nucleus which is a curved band with parallel sides for an appreciable length. Metamyelocytes are not found in the peripheral blood in healthy dogs.

He /

He mentions that the granules of the eosinophil do not usually obscure the nucleus. The granules are described. Occasionally small vacuoles are seen, which are clear cut and rounded.

Basophils are described and he states that the granules often lie over the nucleus.

The small lymphocyte is the commonest form of lymphocyte seen. The nuclear chromatin is clumped and stains deeply. Granules may be present.

Two distinct forms of monocytes are described, and intermediate forms can be demonstrated. Type I has a rounded nucleus, the chromatin of which is lacy almost granular and stains intensely. The cytoplasm is granular and takes a deep blue stain. Pink granules are often seen. Type II is lighter staining, the nuclear pattern delicate, and the outlines often irregular. The nucleus may resemble that of a metamyelocyte or band neutrophil. The cytoplasm takes on a grey blue stain and granules are present. Type II monocytes are seen especially when the monocyte counts are raised.

Photographs are given showing some of these cells but they are unfortunately black and white, not coloured.

No mention is made of other types of cells seen in the blood of healthy dogs.

PRESENT WORK

The blood smears prepared for other parts of this work have been /

have been studied, to identify the types of cells present, and their shape, size and colouration. A total of over nine hundred slides have been examined.

Apart from the sections dealing with special staining techniques such as eosinophils and reticulocytes, all the colours described appertain to cells stained with Leishman's Stain. Colour photographs are included to illustrate many of the cells described.

RESULTS

ERYTHROCYTIC SERIES

The developmental stages and nomenclature used for each stage are the same as those described by Whitby and Britten (1957). Pro-erythroblasts and early normoblasts were never seen in the peripheral blood of healthy dogs. The following stages were seen however.

Intermediate Normoblast

This cell is larger than a mature erythrocyte with a basophilic cytoplasm. The nuclear chromatin is patchy, each dark patch having lighter areas between them. In a few cases they resembled the spokes of a wheel.

These cells were seen in two out of one hundred random samples. They were found slightly more commonly in pregnant bitches nearing parturition. (Four samples out of fifty had intermediate normoblasts). There were never more than two intermediate normoblasts for every four hundred white cells counted. An exception to this is Dog H.14 in which there were 1034 inter-:mediate normoblasts per cu.m.m. at the age of four months. The cause is unknown.

Late normoblasts

This is a smaller cell than the intermediate form with a pyknotic fragmented or partly extruded nucleus. The nucleus is smaller than that of the intermediate normoblast and the cytoplasm has a definite pink colouration. (Fig.17).

These cells are more common than the intermediate form. Out of one hundred random dogs ten had late normoblasts present. The numbers per dog ranged between 32 per cu.m.m. and 100 per cu.m.m. One dog H.14, had very large numbers of late normoblasts present in the blood from the age of four months to two years. The highest value was 7,000 per cu.m.m. This dog remained well and the red cell counts were high. The cause is unknown.

The number of late normoblasts rose towards parturition, being seen in fifteen out of fifty samples from pregnant bitches (20 per cu.m.m. - 3,220 per cu.m.m.). The average was less than 100 per cu.m.m.

In twenty-five samples from dogs aged less than four months, nineteen had late normoblasts present (20 per cu.m.m. - 718 per cu.m.m.). The majority had less than one hundred late normoblasts per cu.m.m.

Reticulocytes

These are non-nucleated red cells which show a reticular network with the cytoplasm after supravital staining with brilliant cresyl purple. (Fig. 8). Cells showing a uniform blue colouration with this stain are not considered to be true reticulocytes.

The /

The incidence of reticulocytes in young dogs has already been described. In pregnant bitches they rose as parturition approached.

In normal adult dogs the average values were 0 - 0.6% of the red cell count.

Erythrocytes

This is a mature non-nucleated red cell (Figs. 8-31). Anisocytosis (Fig.16) and poikilocytosis (Fig. 16) were both seen commonly. The degree of the size variations was small but variations in shape were seen in almost every slide. This is due in some part to the effects of making a smear. It was common to find erythrocytes with clear central areas (Figs. 17, 23 and 29) but true target cells were rare and seen only in young animals or pregnant bitches (Figs. 14 and 16), as was polychromasia (Fig. 16).

Howell-jolly bodies were occasionally seen in young animals or pregnant bitches. (Fig. 19).

THROMBOCYTIC SERIES

Platelets in the dog are usually small with azurophilic granules centrally and a pale blue matrix. (Schalm 1961). It was found that they occurred either singly, or more often in groups. Sometimes these aggregations of platelets were very large. (Figs. 9, 12, 15 and 22). Giant forms occurred frequently but in small numbers only. In a few cases the projections described /

described by Schalm (1961) were seen - usually in smears made after blood-clotting had started. No megalo-karyocytes were seen in the blood of healthy dogs.

LEUCOCYTES

A. NEUTROPHILIC SERIES

Myeloblasts and promyeloblasts were never seen in normal blood.

Myelocytes

These were only seen in one case, that of the bitch with mummified foetuses described in the section on Pregnancy.

Metamyelocyte

The description of this cell is as follows. It is a cell having specific granules in the cytoplasm. The shape of the nucleus varies between that of a bean (Figs. 27 and 28) to that of a thick U-shaped band. (Figs. 9, 27, 29 and 30).

Band Form

My description of this cell differs from that of Schalm (1961). He described it as having a nucleus shaped like a curved or coiled band, with appreciable lengths of the nucleus having parallel sides and a smooth non-indented nuclear membrane. He also states that mature mono-lobed neutrophils often occur in various domestic species and must be differentiated from the true band form.

In /

In this work I have found that these mono-lobed mature neutrophils and band forms occur regularly and I have classified them together as band forms. I consider that they both occur so frequently in normal blood that they are not a sign of abnormality. For this reason my figures for band forms are higher than those of most authors except for those of Irfan (1958) who probably used the same type of classification.

A band form is any neutrophil which has a narrow band shaped nucleus filling approximately one-quarter of the cell. The outline of this band is not important provided there are no definite constrictions. In this case it is classed as a segmented neutrophil. The variations in this type of cell are shown in Figs. 9, 10, 11, 16, 18, 27, 28 and 31.

Segmented Form

This is a neutrophil in which the nucleus shows distinct constrictions, or the nucleus is divided into lobes joined together only by a thin filament. (Figs. 9, 10, 11, 12, 13, 18, 21, 23, 24, and 27 to 31).

Female Projections

The presence of a characteristic nuclear "drum-stick" projection in neutrophils from females was first described by Davidson and Smith (1954). There have been several references since this, and two of the more recent ones from a veterinary viewpoint are those of Irfan (1958) and Schalm (1961).

The /

The shape of the female projection is seen in Fig. 10. Occasionally stumpy projections were seen but these are not classed as true female projections.

Projections identical with those seen in females were seen in two male dogs only. Neither dog had any apparent sexual abnormality present.

Out of a total of two hundred samples from female dogs one hundred and ninety showed the presence of one or more of these "drum-stick" projections.

One hundred neutrophils were examined from each slide for the presence of female projections, and the average number seen in these two hundred samples was 5.4%. The maximum seen from any dog was 16%.

Of the hundred and ninety samples in which female projections were seen, thirty-four had projections on band neutrophils as well as on segmented neutrophils (maximum 3%).

The projections were seen in bitches of all ages from two months upwards, and two bitches which had been speyed several years previously had female projections present (3%). The incidence of female projections did not rise in pregnancy or in bitches which had had previous litters.

B. EOSINOPHILIC SERIES

The eosinophil of the dog has granules which are unequal in shape, size and distribution. Scarborough (1931). These granules can be very large and few in number (Fig. 11) or smaller and /

and numerous (Fig.26). They do not usually obscure the nucleus. The granules do not have a great affinity for acid dyes and stain a reddish-purple in colour.

Vacuoles in the cytoplasm are seen in a few cases (five out of one hundred random dogs). This change is also seen in abnormal dogs. Greyhound eosinophils, however, are different from other dogs, Jones and Paris (1963), Doxey (1963). They regularly show eosinophils with considerable vacuolation of the cytoplasm and small granules (eighty samples out of one hundred showed vacuoles). In some cases the granules were minute and great difficulty was encountered in distinguishing them from degenerating neutrophils. (Figs. 12 and 13).

For this reason a peroxidase method of staining was developed. (Doxey, 1962). This is specific for eosinophils and leaves neutrophils unaffected. The eosinophil granules stain a dark green-black colour and are easily distinguished from neutrophils (Fig. 14). In eosinophils with very small granules the colour reaction is not as distinct as those with large granules.

Pyrogallol reacts with verdoperoxidase within the eosinophil granules and the resulting oxidisation produces brown purpurogalline. The final colour of the granules is obtained when stain is applied to the film. The method used is as follows.

Method /

<u>Method:</u> The following method was used to stain eosinophils in films of canine blood. Only fresh smears were used which had been air dried for thirty minutes.

1. Fix smears in formalin (40% H.C.H.O) - 95% ethyl alcohol, 1:9, 30 sec.
2. Wash in distilled water and air dry.
3. Flood the slide with a mixture consisting of 10% aqueous pyrogallol, 6 ml; and H_2O_2(6% or 20 vols.), 0.1 ml. Allow six minutes for the reaction.
4. Wash in distilled water and air dry.
5. Flood the slide with May-Grünwald stain diluted 1:1 with water. Pour off after two minutes.
6. Flood the slide with Giemsa stain diluted 1:7 with water, eight minutes.
7. Wash well and dry.

Both the stains were diluted with unbuffered glass-distilled water.

Experience has shown that the May-Grünwald Giemsa stain gives the best results, but Leishman's stain (1:2 with water) used in the normal way with a staining time of fifteen minutes also stains the granules and so does Giemsa's (1:2) left on for five minutes. The staining is therefore a matter of individual preference. All stains were manufactured by G.T. Gurr Ltd., London.

Dogs of other breeds living in the same kennel with greyhounds had eosinophils which stained normally and were not vacuolated.

C. /

C. BASOPHILIC SERIES

Basophils were very rare and seen only in one sample out of two hundred random samples. The granules are basophilic and often cover the nucleus.

LYMPHOCYTIC SERIES

Lymphoblast

These are cells of the lymphocytic series having fine nuclear chromatin. One to four nucleoli are present, and the cytoplasm is basophilic (Fig.15). These cells are seen occasionally in the blood of normal dogs, thirteen samples out of one hundred random samples having them present. The largest number seen in any of these hundred samples was 200 per cu.m.m. In most cases only one was seen in a count of four hundred white cells.

The presence of lymphoblasts was more likely to occur in kennelled dogs than in pets. (Twenty-four out of one hundred samples from kennelled dogs as against eight out of one hundred samples from pets).

Prolymphocyte

The nuclear chromatin is coarser than that of a lymphoblast and it is larger than a mature lymphocyte. No nucleoli are present. These cells are seen occasionally in the blood, fifteen dogs from one hundred random dogs having them. Dogs from Kennel S had a higher incidence than other dogs; the reason is unknown but may be associated with antibody production.

Mature /

Mature Lymphocyte

Two forms are seen - a) Small Lymphocytes in which the nucleus occupies almost the whole of the cell and there is little cytoplasm, and b) a Large Lymphocyte which is a bigger cell having a more abundant cytoplasm. In both types the cytoplasm is a pale blue colour and the nucleus dark with a coarse chromatin structure (Figs. 13, 15, 16, 18, 19 and 24).

In some cases the nucleus was very dark and pyknotic while in others it was lighter and uniform in colour. (Figs. 13, 16 and 19).

Variations in both types of lymphocytes occur in normal dogs. Lymphocytes with indented bean-shaped nuclei are seen occasionally. (Fig. 17). One or two such forms were seen while counting the differential white counts in twenty out of one hundred dogs.

Very rarely a bi-nuclear lymphocyte was seen, the nucleus being in two completely separate halves. One dog out of one hundred random dogs showed one such cell.

Mature lymphocytes, both large and small, were occasionally seen to have clear circular areas in the nucleus. (Fig. 18). These resembled nucleoli but showed no distinct demarcation and were not considered to be true nucleoli. It is possible that they are staining abnormalities. Five dogs out of one hundred random samples /

samples had such cells present and in some cases constituted up to ten per cent of the total lymphocyte count. This type of lymphocyte is similar to the "mural type" described by Busch and Van Bergen (1902).

Gum Precht Shadow Cells

These were seen in thirty-one out of one hundred samples. They are lymphocytes which have broken during the preparation of the blood films. (Figs. 18 and 19).

MONOCYTIC SERIES

No monoblasts or pro-monocytes were identified in the blood of normal dogs.

Monocytes of the two types described by Schalm (1961) were seen together with intermediate forms. (Figs. 12, 13, 14, 20, 24 and 26). The cytoplasm in all cases was a blue-grey colour and quite distinct from the cytoplasm of lymphocytes. The nucleus generally had a "lacey" appearance but were occasionally quite dense and resembled the uniformly stained nucleus of a lymphocyte.

Vacuoles were occasionally present in the cytoplasm. The exact numbers were not calculated.

Monocyte-like cells with little or no cytoplasm were seen at the edges of a considerable number of smears. They are probably monocytes ruptured by making the smear. (Fig. 21).

MISCELLANEOUS /

MISCELLANEOUS CELLS

These cells include those which do not fall into the above categories but are seen regularly in the blood of healthy dogs.

Proplasmoblasts and Proplasmocytes (Türk's Irritation Forms).

These cells are the intermediate stages in the production of plasma cells which are closely related to lymphocytes and involved in antibody production. No mature plasma cells were seen in normal blood although very occasionally a cell resembling a plasma cell, but with a smaller amount of cytoplasm, was seen.

Proplasmoblasts are large cells with a large eccentrically placed nucleus, which is often indented. Several nucleoli were visible in the nucleus, and the nuclear chromatin is dark with a reticular appearance. The cytoplasm is deeply basophilic and there is often a clear area, with a reddish colouration round it, close to the nucleus (Fig. 22). These cells were seen in sixteen out of one hundred random samples. They appear more commonly during pregnancy (eleven out of fifty samples) and also in dogs from Kennel S. The highest number seen in any one dog was 170 per cu.m.m.

Proplasmocytes appear identical with proplasmoblasts, but no nucleoli are visible. (Figs. 23 and 24). They vary in shape and can be round or elongated. These cells were seen in twenty-two out of one hundred samples, and again are more common during pregnancy /

pregnancy (twenty-one out of fifty samples) and in dogs from Kennel S. The highest number seen was 420 per cu.m.m. (S.16).

Both types of cell occurred much more frequently in dogs under five months of age. In this class of dog twenty-eight out of fifty samples showed these cells.

Endothelial Cells

These cells occurred infrequently and probably came from the skin puncture wound and are not true blood cells.

The conspicuous nucleus was irregular and had a distinct granular appearance. Often the cytoplasm was absent or smeared due to the cell rupturing when the smears were prepared. The cytoplasm was pale blue or pink.

Unidentified Cells

Some cells were impossible to differentiate accurately, as only one or two cells appeared in the smear and there was no proper indication as to what they might be. An example of such a cell is seen in Fig. 25. One in every ten samples showed the presence of cells classified in this category.

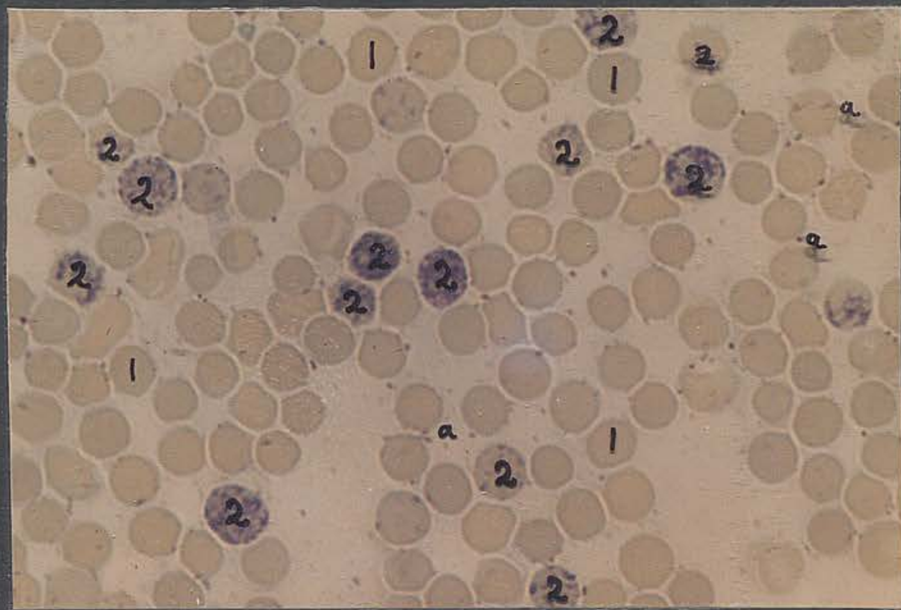


FIG. 8

Case P10 Mongrel male 1 day old
1) mature erythrocytes; 2) reticulocytes; a) stain debris
x 1300

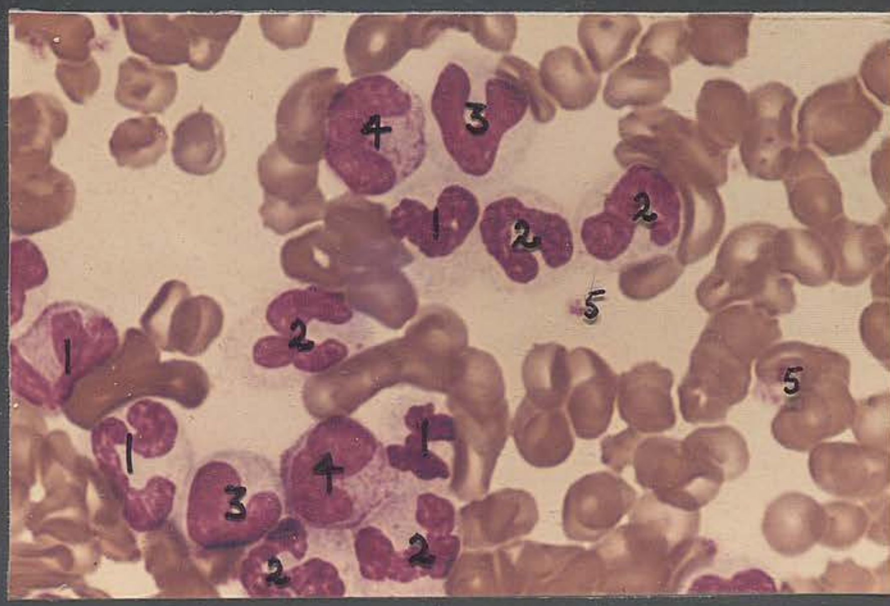


FIG. 9

Case PHI52. Cocker Spaniel Female 10 years (see Pyometra)
1) segmented neutrophil; 2) band form neutrophil;
3) metamyelocyte; 4) metamyelocyte showing distinct granules; 5) platelets. x 1300

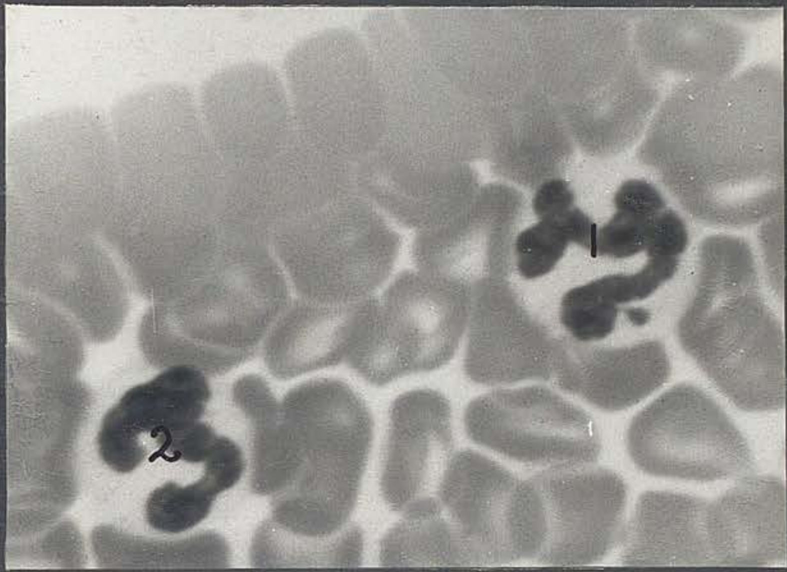


FIG. 10

Case P38 Alsatian Female 2 years
1) mature neutrophil showing female projection; 2) band form neutrophil. x 2400

FIG. 11

Case P.40 Shetland Collie Male 11 months
1) segmented neutrophil; 2) band form neutrophil; 3) eosinophil; 4) ruptured eosinophil showing granules of differing sizes. x 1300

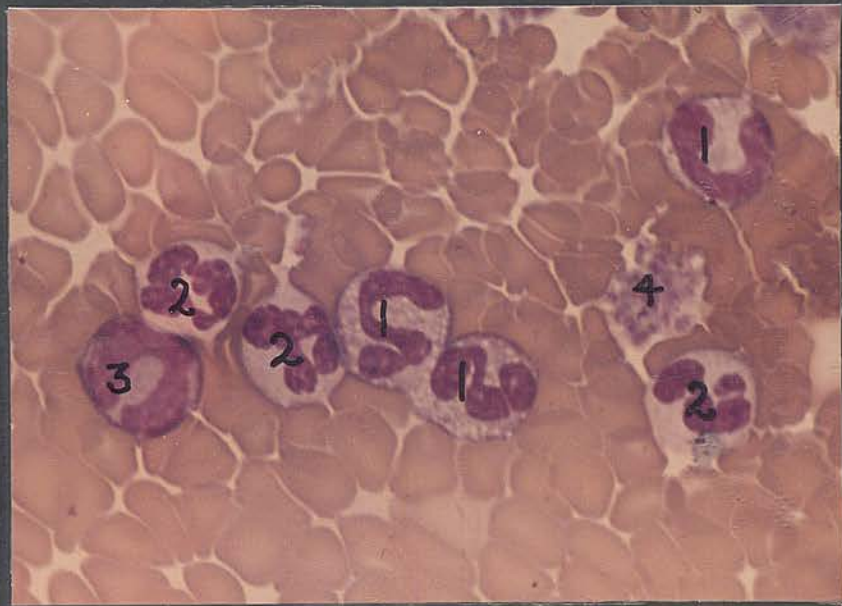


FIG. 12

Case C8h Greyhound Male 1 year
1) Greyhound eosinophil showing vacuolation; 2) segmented neutrophil; 3) Type II. monocyte; 4) bunch of platelets
x 1300

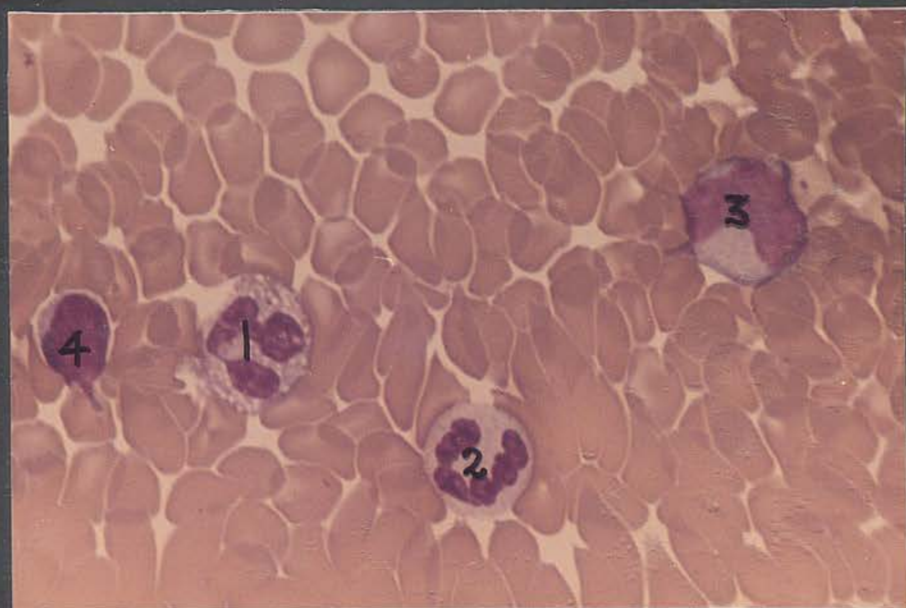


FIG. 13

Case C8h Greyhound Male 1 year
1) Greyhound eosinophil showing vacuolation; 2) segmented neutrophil; 3) Type II. monocyte; 4) small lymphocyte (ruptured) showing uneven nuclear staining
x 1300

FIG. 14
Case P3 Corgi Female 7 years
1) Type I monocyte; 2) eosinophil (stained pyrogallol)
3) target cell. x 1300

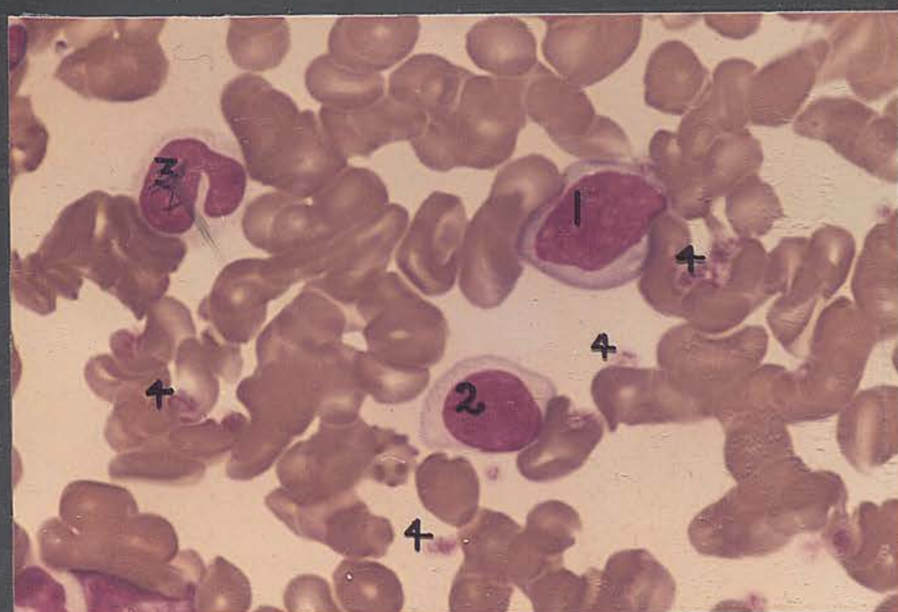
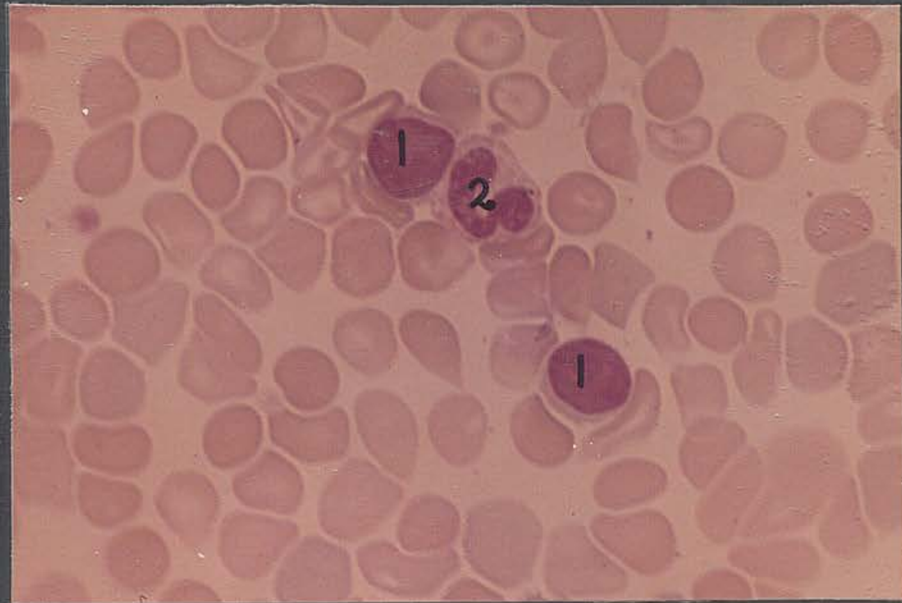
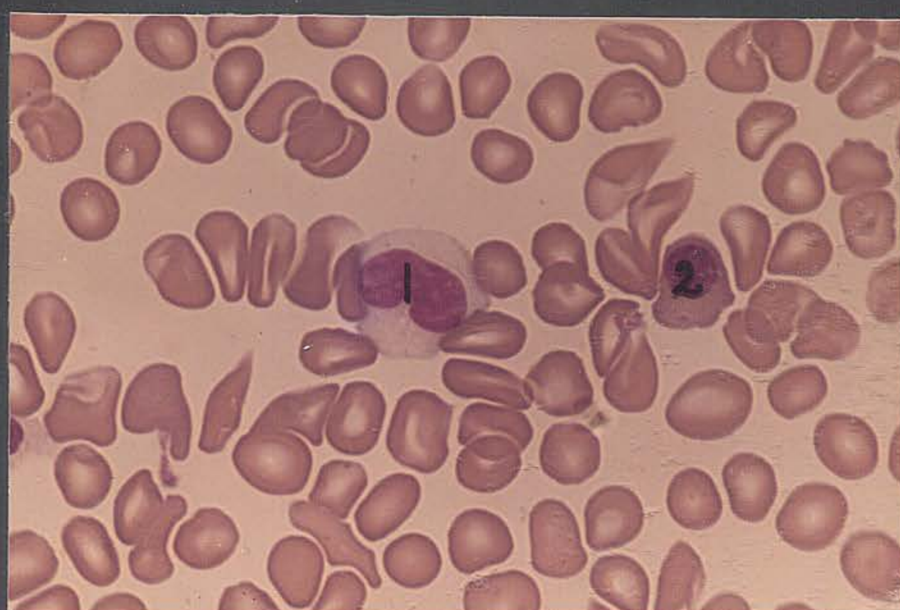


FIG. 15
Case S17d Beagle Female 7 months
1) lymphoblast showing four distinct nucleoli; 2) large lymphocyte; 3) metamyelocyte; 4) platelets. x 1300



<u>FIG. 16</u>

Case C8 Greyhound Male 6 weeks
1) small uniformly stained lymphocyte; 2) band form neutrophil; 3) target cell. Anisocytosis and poikilocytosis are both seen in this film and also polychromasia.



<u>FIG. 17</u>

Case P4 Greyhound Female 1½ years
1) large lymphocyte with an indented nucleus; 2) late normoblast. The colour variation at the edge of the lymphocyte is due to a camera defect. x 1300

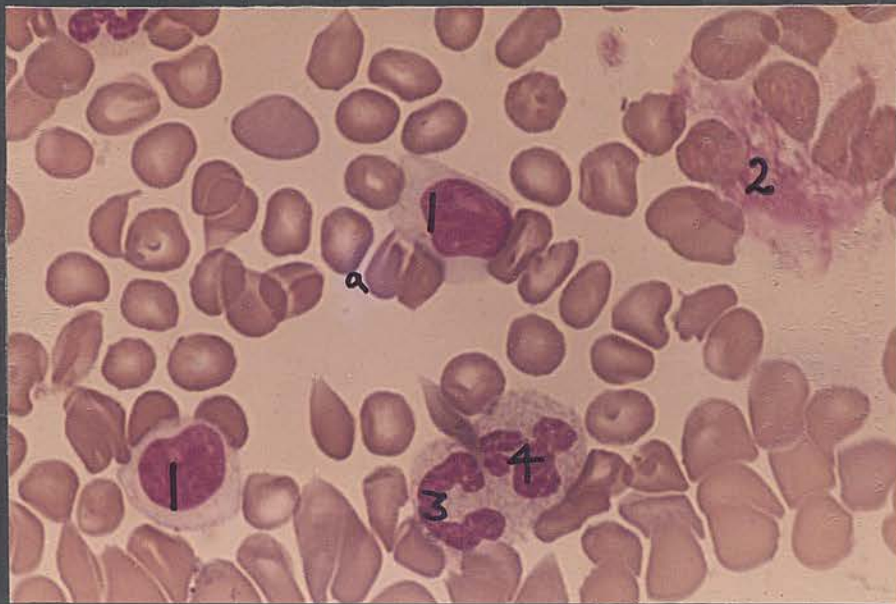


FIG. 18
Case C8b Greyhound Male 14 weeks
1) large lymphocyte showing clear areas in the nucleus which resemble nucleoli; 2) Gum Precht shadow cell; 3) band form neutrophil; 4) segmented neutrophil. a - camera defect.
x 1300

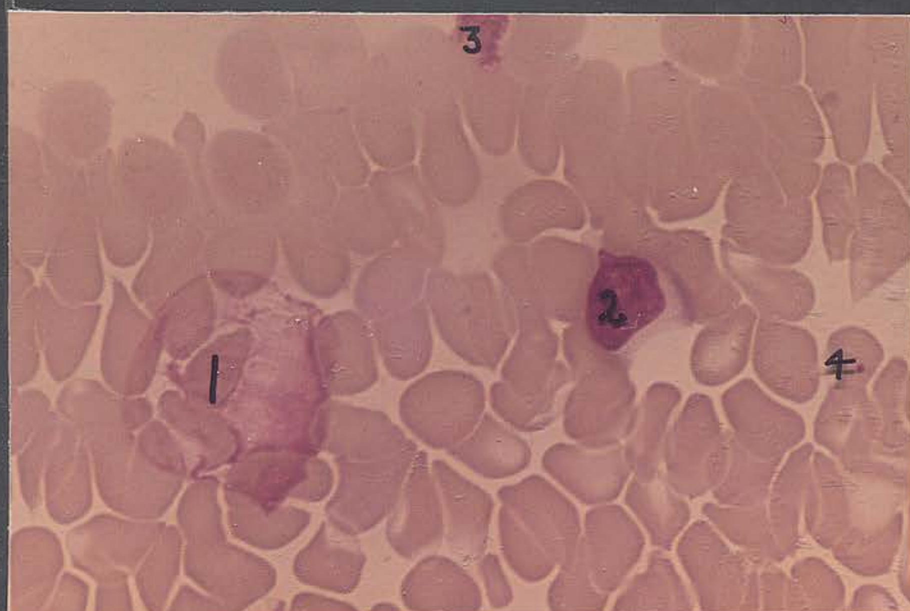


FIG. 19
Case P26 Mongrel Female 1 day
1) Gum Precht shadow cell; 2) small lymphocyte with a pyknotic nucleus; 3) platelets; 4) Howell Jolly body. x 1300

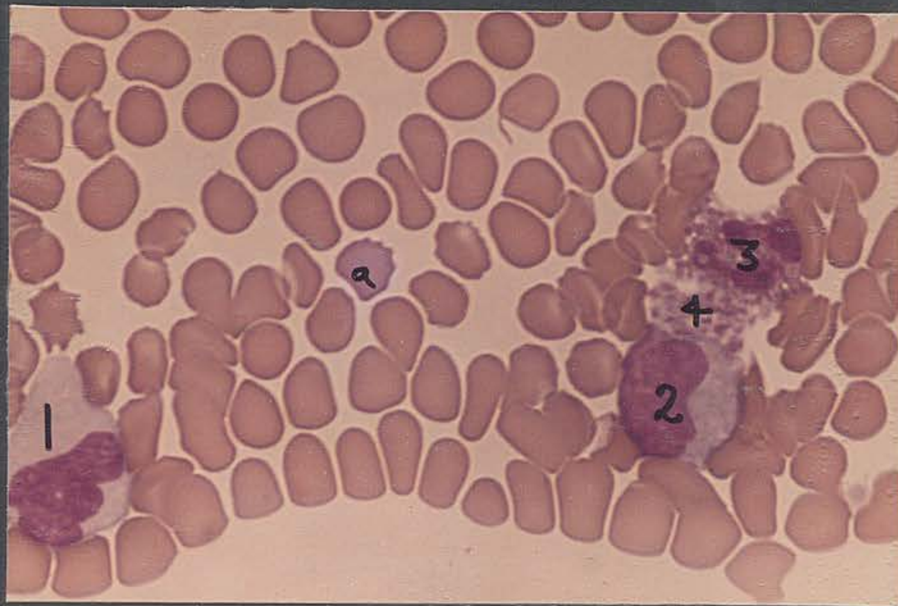


FIG. 20

Case P29 West Highland White Male 2 years
1) Type II monocyte; 2) intermediate monocyte (note grey blue cytoplasm); 3) eosinophil; 4) platelets. a - camera defect
x 1300

FIG. 21

Case P40 Shetland Collie Male 11 months
1) ruptured monocyte; 2) segmented neutrophil (blue edge due to camera defect); 3) platelets. x 1300

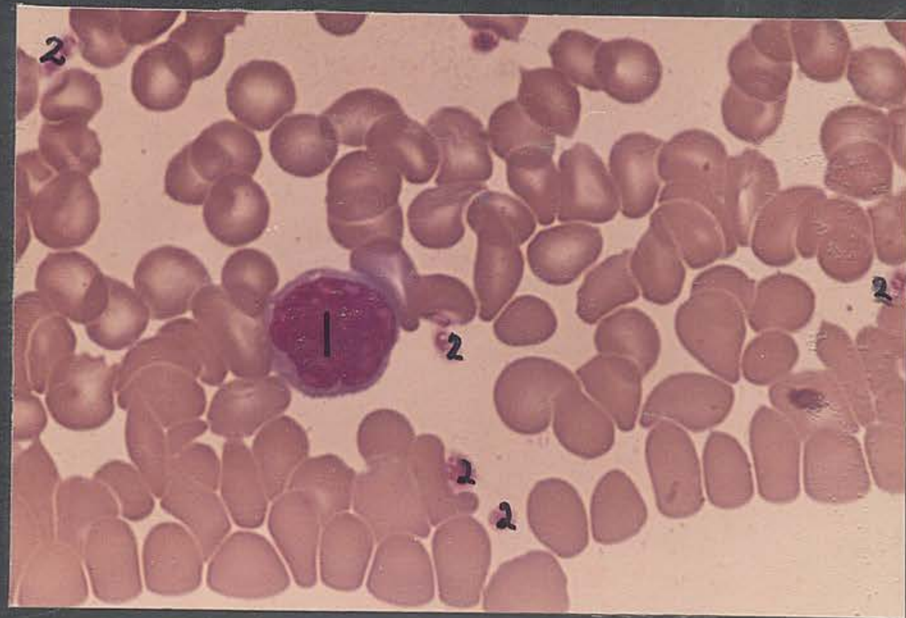


FIG. 22
Case W3 Labrador Male 10 months
1) proplasmoblast showing seven nucleoli; 2) platelets. x 1300

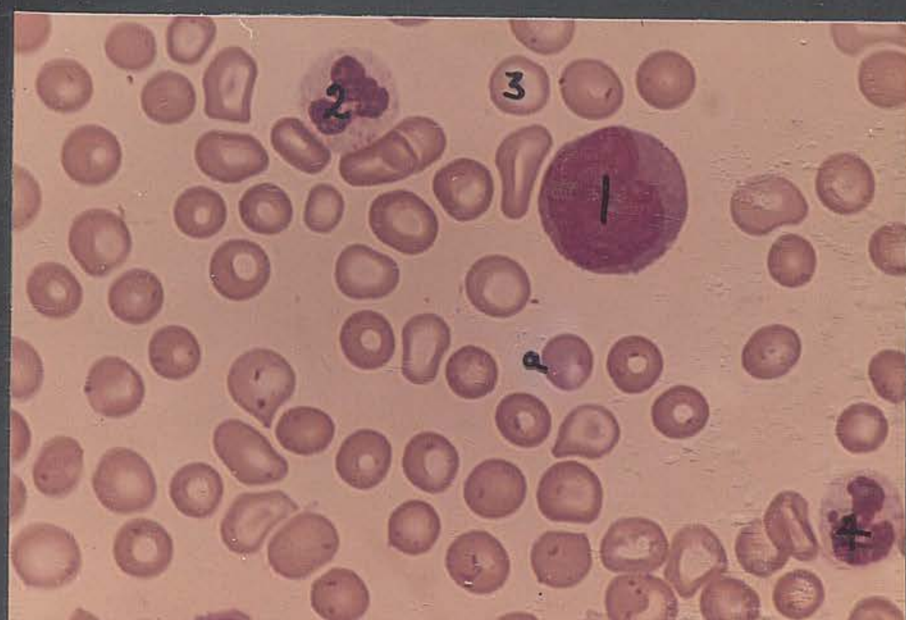


FIG. 23
Case P27 Labrador Male 6 months
1) large rounded proplasmocyte (note lacy appearance of nucleus); 2) segmented neutrophils; 3) target cell; 4) segmented neutrophil with female like projection which is very rare in the male.
a - camera defect. x 1300

FIG. 24

Case S16b Beagle Female 15 weeks

1) elongated proplasmocyte showing basophilic cytoplasm and reddish area round the nucleus; 2) intermediate monocyte; 3) small lymphocyte; 4) segmented neutrophil; 5) platelets. x 1300

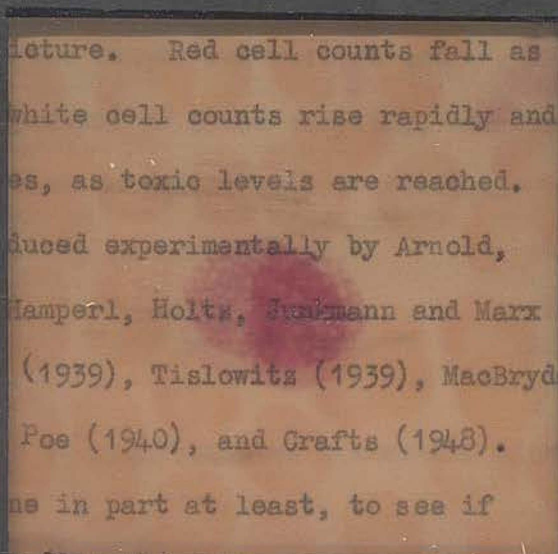


FIG. 25

Case S17d Beagle Female 30 weeks

1) Unidentified cell showing considerable vacuolation x 1950

BLOOD PICTURE OF SOME ABNORMAL DOGS

INTRODUCTION

The previous section dealt with the variations in the blood picture of normal dogs and in that section work was done on pregnancy, false pregnancy and oestrus periods. These three conditions are all associated with hormonal influences.

The two abnormal conditions which will be studied in this section are pyometra and mammary tumours, both of which are also associated with hormonal imbalance.

By studying these conditions it is hoped to obtain a clearer picture of the changes occurring in the blood in two of the many clinical conditions associated with hormonal imbalance which occur in the bitch. Several authors have shown that when oestrogens are given experimentally in large doses violent alterations occur in the blood picture. Red cell counts fall as do platelet counts, while total white cell counts rise rapidly and then fall away to very low figures, as toxic levels are reached. This type of picture has been induced experimentally by Arnold, Holtz, and Marx (1936), Arnold, Hamperl, Holtz, Junkmann and Marx (1937), Tislowitz (1938), Arnold (1939), Tislowitz (1939), MacBryde Castrodale, Helwig, Bierbaum and Poe (1940), and Crafts (1948).

The present study was done in part at least, to see if similar changes occurred when oestrogen imbalance occurred naturally.

Conditions /

Conditions such as hormonal alopecia and anal adenoma which are also associated with hormonal imbalance, were not studied, as it was not possible to obtain enough cases in the time available.

In the various cases of pyometra a clinical diagnosis was made and a blood sample obtained. After this ovariohysterectomy was carried out and the blood picture has been correlated with the state of the uterus as well as with the clinical signs. A few cases diagnosed clinically as pyometra, but which turned out to be suffering from other abnormalities, have been examined, and a comparison made between the blood pictures in these cases and those of true pyometra.

In the cases of mammary tumours the blood picture has again been correlated with the clinical signs and also with the type of tumour. In all cases the tumour was removed and examined histologically. The method of blood sampling, and the materials and methods used to examine the blood are identical with those described in the section on normal dogs.

PYOMETRA

REVIEW OF THE LITERATURE

The literature relating to pyometra does not extend back as far as that on normal dogs.

In 1939 De Vita wrote a preliminary report on pyometra. He deduced that it is directly related to hormonal imbalance and bitches /

bitches suffering from pyometra often have histories of irregular oestrus periods, breeding failures, false pregnancies, and mammary tumours. He stated that until the condition was produced experimentally his theory could not be established.

The condition was found to occur most frequently in bitches of six years of age and over. (Twenty-four cases examined). In seven cases he found sterile pus in the uterus and only in three cases were organisms found (coliforms, streptococci and staphylococci). He did not give any information on blood changes.

In 1940 a considerable amount of literature appeared concerning pyometra. Engle (1940) considered true pyometra to occur only when the cervix was closed and the uterus filled with pus. He does not include simple endometritis. The age incidence he found to be four to twelve years. This paper was given at a congress and in the discussion which followed Crawford stated that the red cell counts were low in most cases of pyometra and that the white cell count varies from 18 - 25000 per cu.m.m. Haigler said that he could diagnose the condition using a blood picture and found the white cells to be 40,000 per cu.m.m. or over. Jones states that an increase in white cells was variable but that there was always a shift to the left.

Khuen, Park and Adler (1940) investigated sixteen cases of pyometra, twelve cases of metritis, and two cases of false pregnancy. Their average results and extremes of variation are as follows:

Pyometra

	R.B.C. millions per cu.m.m.	W.B.C. per cu.m.m.	Stab. %	Juv. %	Lymph. %
Average	5.176	42,500	35	3	14
Range	0.213-8.780	22800-81500	10 - 55	0 - 8	5 - 30

Metritis

Average	5.55	12,300	17	0	20
Range	3.860-9.000	6400-19700	6 - 28	0 - 4	6 - 36

They considered that the total white cell counts were always raised and that there was a shift to the left. The number of lymphocytes fell (they gave only percentage figures). The recovery rate was better if the white cell counts were low.

Haigler and Hawkins (1940) discussed the use of the blood picture as an aid to the diagnosis of pyometra, although they made no attempt to correlate the blood picture with the type of pyometra. They found the average white cell count from the twenty-four cases studied to be 50,000 per cu.m.m. The neutrophils were large, stained evenly showed toxic granulation and had a smoothly contoured nucleus. They considered that the presence of this type of cell was diagnostic of pyometra and thought its presence more help as a diagnostic aid than an elevated white cell count. To prove this they cited the case of a spaniel showing the following blood picture. As it did not have any of the characteristic neutrophils present, they did not diagnose it as a case of pyometra.

W.BBCC per cu.m.m.	Juveniles	Stabs.	Seg.	Lymph.	Mono.
24,950	3%	39%	54%	2%	2%

This bitch turned out to be pregnant and whelped normally two days later.

The average results and ranges of variation given for the pyometra cases, whose ages varied from four to twelve years, are as follows:

	Average	Range
W.B.C. per cu.m.m.	50,022	22,100 - 104,000
Myelo. %	0.15	0 - 4
Juvenile %	6.9	0 - 16
Stab. %	57.3	39 - 70
Seg. %	23.4	8 - 41
Lymph. %	8.9	1 - 16
Mono. %	3.4	0 - 11
Eosin. %	0.4	0 - 6

Morris, Allison and White (1942) describe both open and closed cases of pyometra, and the appearance of the uterus. They found that there was a definite shift to the left and that a characteristic stab cell was seen in cases of pyometra. This cell was larger than normal with a U-shaped nucleus. In closed pyometra there was a marked leucocytosis.

Their results were obtained from twelve dogs aged between two and a half and twelve years old.

The /

The following results are given:

per cu.m.m.	Closed Pyometra	Closed pyometra six days after ovariohysterectomy	Open Pyometra
W.B.C.	77,010	18,950	26,125
Seg.	38,474	12,555	13,889
Lymph.	6,430	2,465	2,889
Stab.	26,797	3,600	8,509
Eosin.	117	190	262
Mono.	3,377	47	272
Baso.	225	47	-
Juv.	1,487	47	-
Myelo.	103	-	301

Bloom (1944) studied both the blood picture and the bone marrow in cases of pyometra. He found that there was often an increase in red cells and haemoglobin due to dehydration. The white cell counts rose and there was a shift to the left. There was disturbed maturation of neutrophils, and the non-lobulated neutrophils showed toxic changes. Lymphocytes had a tendency to form pseudopodia.

From five well advanced cases of pyometra the average values and ranges of variation are as follows:

	Average	Range
R.B.C. millions per cu.m.m.	7.932	7.12 - 8.92
Hb. gms/100 mls.	15.1	14.1 - 16.2
W.B.C. per cu.m.m.	99,480	48,800 - 16,6400
Myelocytes %	1.6	1 - 3
Non.lob. %	58.6	43 - 76.5
Seg. %	30.9	11.5 - 49
Eosin. %	0.4	0 - 0.5
Mono. %	3.8	1.5 - 6.5
Lymph. %	4.5	1 - 7
Pathological Lymphs. %	0.2	0 - 0.5
Normoblasts/100 w.b.c.s	0.3	0 - 1.5

Lacroix (1947) studied twenty cases of pyometra and discussed clinical signs and treatment.

With regard to the blood picture he says that the number of non-lobulated neutrophils varied with the clinical picture but he found them as high as 50% of the white count. (No absolute figures given). He found the following values.

	Average	Range
R.B.C. per cu.m.m.	4,767,000	2,750,000 - 6,600,000
W.B.C. per cu.m.m.	27,360	10,500 - 101,000
Lymph. %	18	5 - 38

This is the first author to give a white cell count within the normal range in cases of pyometra although he gives no details of the cases clinically.

He /

He gives the full blood picture in five cases of which two had white counts within the normal range. These two results are given here:

	Case I.	Case II.
R.B.C. millions per cu.m.m.	2.75	6.6
Hb. gms/100 mls.	3.3	13.2
W.B.C. per cu.m.m.	10,200	12,500
Neut. %	92	68
Seg. %	52	60
Stab. %	37	8
Juvenile %	0	0
Myelo. %	3	0
Eosin. %	0	3
Lymph. %	8	28
Mono. %	0	1
Normoblast %	4	0

Case II. shows an almost normal blood picture while Case I. has a degenerative shift to the left.

Baier, Kalich and Taxacher (1953) investigated one hundred and ten cases of pyometra but give no blood pictures. In only nineteen cases were bacteria isolated from the uterus and in eighteen of these the organisms were coliforms. They consider this type of organism to be commensals as they can be isolated from faeces.

In 1954 Rehfeld in an article describing the work of other authors says that there is a leucocytosis due to an increase in /

in neutrophils in cases of pyometra. Toxic granulation is often present. Anaemia occurs but may be masked by haemoconcentration. In all cases the sedimentation rate is increased, and lies in the region of 10 - 30 m.m. per hour.

Low (1954) described the clinical signs and pathology of cystic hyperplastic endometritis but gives no blood pictures.

Knowles (1955) gives the following average result for cases of pyometra. White cell counts 30,000 per cu.m.m.

Dow (1957) described the various types of uterine lesions seen in cases of pyometra. He described four distinct types and examined one hundred cases.

Type I is uncomplicated cystic hyperplasia with a slight discharge during metoestrus. Twenty-three cases had this type and E. coli was isolated from six of these cases in metoestrus. The ages ranged from three to twelve years.

Type II is cystic hyperplasia with vaginal discharge and a slight leucocytosis. Seventeen cases aged from four to eleven years fell into this group and of these fourteen cases had E. coli isolated from them. The red cells and haemoglobins were normal, the white counts 12 - 15,000 per cu.m.m. with normal differential counts.

Type III is acute endometritis and cystic hyperplasia. The cervix can be either open or closed and the animal is clinically /

clinically ill and shows a leucocytosis. Forty-nine cases, aged four to thirteen years, fell into this group. E. coli was isolated from four cases, coagulase positive staphylococci from six cases and B-haemolytic streptococci from five cases.

The white cell counts varied from 19 - 145,000 per cu.m.m. and there was a regenerative shift to the left. Toxic granulation and vacuolation was seen in the non-lobulated neutrophils. In the cases with a closed cervix the white cell counts were always above 50,000 per cu.m.m. The E.S.R. varied from 10 - 55 m.m. per hour and if the value was over 30 m.m. per hour the prognosis was poor.

Type IV, in which there were eleven cases, is chronic endometritis. The degree of illness varied with the degree of patency of the cervix. The same organisms were isolated as for Type III, eight cases having E. coli present.

In cases with an open cervix the white counts varied from 16 - 21,000 per cu.m.m. and in cases with a closed cervix from 31 - 68,000 per cu.m.m. He noted the presence of bizarre "star cell" monocytes. The E.S.R. varied from 8 - 40 m.m. per hour and was highest in closed cases.

He showed that a history of false pregnancies or irregular oestrus periods was common in bitches with pyometra. Most cases were nulliparous but a total of twenty-five had had one or more litters several years previously.

Dow /

Dow showed quite clearly that pyometra is associated with stilboestrol-progesterone imbalance and was able to produce cases experimentally by daily injections of stilboestrol diproprionate followed by progesterone. These injections were repeated in cycles and if the progesterone dose was raised during the sixth cycle clinical cases of pyometra resulted.

Irfan (1958) in his Ph.D. thesis examined thirty-nine cases of pyometra. The ages varied from four to fifteen years, the majority occurring at about eight years of age. He classed his results into three groups as follows:-

Group I. Open pyometra - 16 cases.

In this type he obtained the following results:-

Red cells millions per cu.m.m.	2.95 - 6.80
Haemoglobin gms/100 mls.	7.9 - 17.9
P.C.V. %	24 - 60
W.B.C. per cu.m.m.	15,000 - 99,100

In the majority of cases the white cell totals were below 40,000 per cu.m.m. One case was within the normal range and all cases showed a shift to the left. Monocytes were increased in many cases and lymphocytes in four cases. The sedimentation rate was increased in six out of thirteen cases and varied from 0 - 69 m.m. per hour.

Group II.

This consisted of fourteen cases of closed pyometra.

The /

The blood pictures were as follows:-

Red cells millions per cu.m.m.	3.4 - 6.42 (six cases anaemic)
Hb. gms/100 mls.	8.7 - 19.2
P.C.V. %	30 - 56
W.B.C. per cu.m.m.	18,500 - 68,300

The majority of cases had white counts above 40,000 per cu.m.m. and all showed a shift to the left. Lymphocytes were increased in seven cases and monocytes in eleven cases. The sedimentation rates varied from 1 - 30 m.m. per hour.

Group III.

This group consisted of four cases of cystic hyperplasia. The blood pictures were as follows:-

Red cells millions per cu.m.m.	3.5 - 7
Hb. gms/100 mls.	9 - 19.5
P.C.V. %	33 - 68
W.B.C. per cu.m.m.	16,500 - 25,900 (All showed a shift to the left).

Sedimentation rates were 1 m.m. per hour in two cases.

He examined two cases of pyometra eleven days after ovariohysterectomy and found that the white counts had fallen - 19,500 - 17,400 and 46,900 - 18,400 respectively. No attempt was made to type the uterine lesions and no bacteriological examination was carried out.

Talanti (1959) discussed the reasons for increased thirst in cases of pyometra. He found the kidneys to be unaffected but that the amount of neurosecretory material decreased in some parts of the hypothalamic-hypophysical system in cases of pyometra.

Christoph /

Christoph (1961) showed that out of forty-two cases of pyometra fourteen per cent had normal sedimentation rates, thirty-one per cent had increased rates and fifty-five per cent had markedly increased rates.

Out of three hundred and sixty-three cases of pyometra he obtained the following results, given as percentages:-

363 blood pictures

	W.B.C. less than 8,000 per cu.m.m.	8 - 10,000 W.B.C. per cu.m.m.	10 - 30,000 W.B.C. per cu.m.m.	Over 30,000 W.B.C. per cu.m.m.
	7%	1%	41%	34%
Shift to the left	1%	0	15%	21%
Normal	6%	1%	20%	12%
Shift to the right	0	0	6%	2%
R.B.C. less than 5.5 million	2%	0	14%	22%
R.B.C. normal	5%	1%	27%	12%
R.B.C. over 8 million	0	0	0	0
B.S.R. normal	3%	0	12%	6%
B.S.R. raised	2%	0	9%	4%
B.S.R. markedly raised	1%	1%	14%	20%

He also noted the eosinopenia occurred in a considerable number of cases.

Rieck /

Rieck (1961) in an article dealing mainly with the causes, pathology and diagnosis of pyometra says that two cases of perforated pyometra with peritonitis had normal sedimentation rates and only a small rise in white cells and slight shift to the left.

Ewald (1961) found a breed incidence with regard to the three hundred and ninety-five cases he examined. Collies (34%) and Irish setters (28%) had the highest incidence and Poodles (8%) and Dachshunds (3%) the lowest. The dogs were all over five years of age and the majority were seen about four weeks after the last oestrus period.

The blood pictures showed the following results:-

P.C.V. % 18 - 59 (Mean 44)
W.B.C. per cu.m.m. 5,500 - 127,000 (Mean 36,650).

Twenty out of one hundred and fifteen cases had white counts of less than 20,000 per cu.m.m. and in 70% of cases there was a shift to the left. All the cases with white counts of less than 20,000 per cu.m.m. had a vaginal discharge (open cases). Polydipsia was seen in two hundred and thirty-seven of the dogs examined.

Schalm (1961) gives a considerable amount of information on the blood picture in pyometra. He describes the uterine conditions and clinical signs by the side of each blood picture. In /

In closed cases (seven) the white counts varied from 26,800 to 126,000 per cu.m.m. and the packed cell volumes from 33 to 50%. In open cases the white counts varied from 14,350 to 112,000 per cu.m.m. and the packed cell volumes from 20 to 53%. Of the eighteen cases described, eleven had a shift to the left (both open and closed cases). The most marked was a dog with a white count of 26,560 per cu.m.m. with 8% myelocytes, 53% metamyelocytes and 21% band forms. Two cases with white cell counts of 14,000 per cu.m.m. had raised neutrophil counts but no shift to the left.

Benjamin (1962) describes the blood changes in pyometra but these are results compiled from the work of authors already reviewed.

Churchill Frost (1963) dealing with pyometra and mammary tumours showed that these conditions are bound up with irregular oestrus periods, and false pregnancies. He gives considerable data on age incidence.

REVIEW OF THE LITERATURE ON PYOMETRA

The work reviewed is extensive and gives an almost complete picture of the blood changes liable to occur in pyometra. The earlier workers deal mainly with the leucocytosis which occurs and stress that a shift to the left is always seen. The presence of cells which are characteristic, from cases of pyometra, are described by three authors. Lacroix (1947) is the first author to describe cases with a normal white cell count but he gives no clinical details of these partiéular cases.

Baier /

Baier, Kalich and Taxacher (1953) describe the bacteriological findings in their cases but give no blood pictures, while Rehfeld (1954) is the first author to describe increases in sedimentation rates in pyometra.

Dow (1957) typed the various forms of pyometra for the first time, and gives details of bacteriological findings. His blood pictures for these various types are not given in full and he does not describe a clinically ill case with a normal white cell count.

Irfan (1958) gives full details of the blood pictures obtained but does not correlate these with the clinical findings very accurately.

Christoph (1961) shows that low white cell counts occur in pyometra but fails to describe the cases from which these results were obtained. He shows clearly that the red cells and sedimentation rates are altered more markedly in cases with high white cell counts.

Schalm (1961) although giving individual results adds little to the existing literature with regard to the correlation of the blood picture with the clinical signs.

As the author had seen cases of pyometra with apparently normal white cell counts it was decided to investigate the blood picture in pyometra again, to see if similar cases could be recorded. The author also wished to correlate the blood picture more closely with the clinical signs and bacteriological examination of the uterine /

uterine contents to see if additional information could be added to the existing literature. It was considered that the use of the blood picture as an aid to differential diagnosis should also be investigated. This particular topic has not been investigated fully before.

PRESENT WORK ON PYOMETRA

The dogs used in this study fall into the category of Pets, and they all came into the Small Animal Hospital at the Veterinary School as clinical cases. These dogs later underwent ovariohysterectomy and the uterus was then examined macroscopically and swabs from the uterine contents taken for bacteriological examination. Where possible the progress of the case was observed, and in a few cases serial blood samples were obtained.

The complete list of dogs examined is given in the Appendix, pages 63 and 63a.

The code number given to each dog is P.H.I. and this is then followed by the individual number for each dog in the same way as for the normal dogs.

Method of investigation of a case of Pyometra

The dog when presented in the clinic was examined and the clinical signs noted. A full history regarding the present illness, and also a past history was obtained whenever possible. A blood sample was removed and examined, using the same techniques described in the section dealing with normal dogs.

After /

After ovariohysterectomy the uterus was weighed, measured and the uterine contents examined. The condition of the uterine lining was ascertained and the ovaries examined.

A bacteriological sample was obtained with a sterile swab pushed into the uterine contents through a hole cut in the wall of the uterus. This was done before the uterus was opened and the contents examined.

Classification of the types of Pyometra

The pyometras were classified according to the system of Dow (1957). No histological examination was carried out in the majority of cases. The acute and chronic pyometras were differentiated on the basis of the age of the animal, the period between the last oestrus and the onset of clinical signs, and the condition of the uterus. This differentiation was carried out with the help of J.C. Whitney (1964).

The letters A and B were used to classify open and closed cases, e.g. Acute open endometritis is classified as Dow, Type III.A.

RESULTS

The results for each dog are given in the Appendix, pages 64 - 68a.

The clinical description of each animal will be briefly given in the text, and these clinical signs will be correlated with the blood picture.

I. /

I. OPEN PYOMETRA - Dow type II.

This dog (PHI.20) had repeated false pregnancies and was treated with stilboestrol on each occasion. The last oestrus was ten weeks before and stilboestrol was given for a false pregnancy. Clinical signs of pyometra developed a week later. The bitch was slightly off colour for two weeks and developed a bloody vaginal discharge three days before being seen.

The uterus was of the Dow type II with numerous cysts and considerable congestion. Bacteriological examination revealed alpha- and beta- haemolytic streptococci in the uterine contents. The main parts of the blood picture are given in the Appendix, page 64. This shows a slight leucocytosis with a raised neutrophil count, but no shift to the left. In all other respects the blood picture was within normal limits.

II. NYMPHOMANIA-LIKE SYNDROME - Dow type II.

Three cases were seen and the relevant parts of the blood pictures are given in the Appendix, page 64. They only showed uterine changes which were like those seen in mymphomania. Age incidence was 5 - 10 years.

The clinical signs are as follows:-

Code No.	Date of last Oestrus	Breeding History	Discharge and Clinical Signs.	Uterus	Bacteriological Examination
PHI.23	Two months before	Numerous false pregnancies	Slight bloody discharge. False pregnancy otherwise normal	Small, congested Numerous cysts	Beta-haemolytic streps. and coliforms
PHI.43	Two months before	Apparently normal	Profuse bleeding from vulva six weeks after oestrus only	Normal size, congested and haemorrhagic. Cysts present.	Sterile

Code No.	Date of last Oestrus	Breeding History	Discharge and Clinical Signs	Uterus	Bacteriological Examination
PHI. 110	Two months ago	Normal 1 litter 3 years ago	Bloody vaginal discharge since oestrus Excess thirst No other signs	Normal size congested cysts present.	Coliforms, Staphylococci and gram-positive cocci

The results indicate that the red cell counts may be slightly lowered due to blood loss but not enough to be significant. The white cell counts are normal and the only other change is that the platelet counts tend to be higher than usual, but are within the normal range.

ACUTE PYOMETRA - Dow type III.

This type can be sub-divided into open and closed cases. A total of thirteen cases were seen. Of these eight were open and five closed cases. The average age was 7 years and the range 3-11 years.

It is well known that the cervix can open and close during the course of the condition, so that an open pyometra can become closed and vice versa. Some of the cases described started as closed cases but then became open ones so the complete clinical history will be given. In this way the changes seen in the blood picture can be correlated with the various stages in the opening or closing of the cervix.

The clinical history for each case will follow and the blood pictures can be found in the Appendix, page 65.

CLOSED /

CLOSED CASES

Code No.	Date of 1st Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.10	10 months ago	Slight discharge for one week. Dull, off food, thirsty, pyrexia for one week	-	-
PHI.10a	-	Discharge ceased eating a bit dehydrated	-	-
PHI.10b	-	No change. Vomited once.	Large endometrium necrotic. 350 cc. of pus in uterus	Non-haemolytic streptococci
PHI.15	6 weeks ago	Hormonal alopecia Off food three weeks; thirst; vomiting	-	-
PHI.15a	-	No change	-	-
PHI.15b	-	Pyrexia, anorexia No discharge. Thirst.	Large endometrium A little necrotic. 650 cc. of pus in uterus	Beta-haemolytic streptococci

This dog died three days after ovariohysterectomy. (Nephritis also)

Code No.	Date of 1st Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.79	4 weeks ago	Ill three weeks; anorexia; thirst and difficulty in breathing	Very large endometrium completely necrotic. 1150 cc. of pus	Coliforms

This died died the day after ovariohysterectomy

CLOSED CASES

Code No.	Date of 1st Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.98	4 days ago	Ill two days; excess thirst	-	-
PHI.98a	-	Thirst the only clinical sign	-	-
PHI.98b	-	Thirst; anorexia vomiting. Started to discharge six hours before. Ovariohysterectomy.	Large endometrium necrotic in patches. 600 cc. of pus	Coliforms
PHI.98c	-	Much improved; five days post operative	-	-
PHI.104	Unknown	Ill two weeks; very dull; vomiting and excess thirst	-	-
PHI.104a	-	A little better after antibiotics	-	-
PHI.104b	-	Very ill; vomiting anorexia.	Large, necrotic endometrium 250 cc. pus	Not done.

The average values for Dow type IIIB cases are shown in the following table.

Acute Pyometra - Average values and ranges of variation

CLOSED CASES.

Acute Pyometra - Average values and ranges of variation

CLOSED CASES

	Average	Range of Variation
R.B.C. millions per cu.m.m.	5.942	4.810 - 6.850
P.C.V. %	42.5	35.5 - 47.0
Hb. grms/100 mls.	15.7	13.1 - 18.4
Sed. Rate m.m. per hour	6.9	1 - 30
W.B.C. per cu.m.m.	33076	11150 - 71750
Myelo. per cu.m.m.	33	0 - 342
Meta. per cu.m.m.	3274	0 - 13356
Band per cu.m.m.	6153	2333 - 19740
Seg. per cu.m.m.	18980	8797 - 37570
Lymph. per cu.m.m.	2183	725 - 3946
Mono. per cu.m.m.	2302	202 - 5830
Eosin. per cu.m.m.	99	0 - 389
Misc. per cu.m.m.	50	0 - 292

These results show that the red cell counts are usually well within the normal range, and only in one sample was the red count less than 5,000,000 per cu.m.m. There is a tendency for the values to fall the longer the condition is present (PHI.98, Appendix page 65). Fluctuations occur if dehydration becomes apparent (PHI.10, Appendix page 65).

The sedimentation rates are usually raised but vary considerably from case to case. (1 - 30 m.m. in one hour).

The length of time the animal has been showing clinical signs does not appear to have any correlation with the sedimentation rates. Cases PHI.15 and PHI.79 had both been ill for three weeks and their sedimentation rates were 13 and 4 mm./hour respectively. Case PHI.98b, on the other hand, had a sedimentation rate of 26 m.m. per hour after an illness of eleven days.

The /

The average white cell count for all samples was 33076 per cu.m.m. with a range of 11150 - 71750 per cu.m.m. The total white cell count raised to varying degrees but they are not always highest in dogs which have been ill for a long time. Case PHI.79 and PHI.15 had both been ill for three weeks and had counts of 71750 and 17600 per cu.m.m. respectively. Case PHI.15a had a normal white count.

The total white cell count did not increase markedly following closure of the cervix in PHI.10.

A shift to the left was seen in eleven out of thirteen samples. The degree of shift to the left was not always associated with the increase in total white cell counts.(PHI.10 had a more marked left shift than PHI.79).

Monocytes were raised in all instances and eosinopenia was seen in three out of five cases. Female neutrophils were reduced in four cases.

The type of bacterial flora in the uterus had no apparent effect on the blood picture.

OPEN CASES - Dow Type III.

The clinical histories and the findings following examination of the uterus are given below. Results are given in the Appendix, page 66.

Code No.	Date of 1st Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.22	3 weeks ago	Ill 3 weeks. Thirst. Anorexia. Dull. Slight yellow vaginal discharge. Has mammary tumour. (Fibroadenoma)	Large, right uterine horn endometrium more necrotic than left. 650 cc. of pus mainly in left horn of uterus	E. coli
PHI.22a	-	Six days post operative recovery slow.	-	-
PHI.25	$2\frac{1}{2}$ months ago	Ill one week. Anorexia. Dull; thirst. Dirty vaginal discharge for one week, now reduced	Small. 200 cc. of pus in uterus. Right horn less necrotic than left	Coliforms
PHI.36	4 weeks ago	Ill six days. Anorexia. Vomiting. Purulent vaginal discharge for four days	Small. 30 cc. of pus. Endometrium completely sloughed and abscessed	Coliforms

Code No.	Date of 1st Oestrus.	Clinical Signs	Uterus	Bacteriological Examination
PHI.71	10 days ago	Ill one week; dull; anorexia No thirst. No discharge	-	-
PHI.71a	-	Profuse vaginal discharge for one day	Small. 20 cc. of pus. Endometrium completely eroded. Ovarian tumour.	Bacilli of the para-colon type.
PHI.105	-	Thirst for 36 hours. Vomiting; Slight vaginal discharge.	-	-
PHI.105a	-	No change	Uterus large and necrotic	
PHI.106	4 weeks ago	Mammary tumour. Ill one week. Thirst, a bit off food. Bloody vaginal discharge for one week	-	-
PHI.106a	-	No change	Uterus moderate in size. Necrotic	
PHI.107	1 month ago	Ill five days. Excess thirst. Foul pink vaginal discharge for one day	-	-
PHI.107a	-	No change	Moderate size. 60 cc. of pus. Not necrotic	Coliforms

Code No.	Date of 1st Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.108	None for three years.	Ill two days. Vomiting and excess thirst. Copious vaginal discharge for two days	-	-
PHI.108a	-	No change	Small, lobulated endometrium, congested and necrotic. 20 cc. of pus	Coliforms

The average results from Dow type IIIA cases are given in the following table. The individual results are given in the Appendix page 66.

OPEN CASES	Average	Range of Variation
R.B.C. millions per cu.m.m.	6.057	4.930 - 7.270
P.C.V. %	46.1	37.5 - 55.0
Hb. grms/100 mls.	17.0	13.7 - 20.0
Sed. Rate m.m. per hour	10	2 - 45
W.B.C. per cu.m.m.	19,088	7100 - 38,000
Myelo. per cu.m.m.	8	0 - 100
Meta. per cu.m.m.	1773	154 - 4347
Band per cu.m.m.	2450	927 - 3865
Seg. per cu.m.m.	11176	2302 - 27374
Lymph. per cu.m.m.	2311	880 - 5080
Mono. per cu.m.m.	1156	0 - 2292
Eosin. per cu.m.m.	181	0 - 697
Misc. per cu.m.m.	33	0 - 301

The red cell counts were within the normal range in all but two cases.

Sedimentation /

Sedimentation rates were raised in all instances but varied widely (2 - 45 m.m. in one hour). Only one sample out of nine had a rate greater than 12 m.m. in one hour. As in closed cases the increase in sedimentation rate had no correlation with the length of illness. PHI.25 and PHI.107a had both been ill for one week and their sedimentation rates were 45 and 6 m.m. in one hour respectively.

The average white cell count for all dogs was 19,088 per cu.m.m. with a range of 7,100 - 38,000 per cu.m.m. This is lower than for closed cases. The degree of elevation of the total counts was not related to the length of illness after the first week. Case PHI.22 had been ill for three weeks while PHI.36 had only been ill for six days, and they had counts of 24125 and 36000 per cu.m.m. respectively. In cases with a profuse vaginal discharge the total white counts were low in the initial stages of the illness (PHI.108).

The total white cell counts tended to rise during the early stages of the condition. (PHI.105, PHI.107 and PHI.108). In the case of PHI.108 the count almost trebled itself in two days.

In four samples the total white cell counts were normal (duration of illness varied from thirty-six hours to one week).

A shift to the left was seen in all of the thirteen samples but it varied from case to case. Case PHI.71 had only a fractional shift to the left and a normal white count, and the case /

case was closed when this first sample was taken. Cases with high white counts did not always show a marked shift to the left (PHI.36) while cases with low white counts sometimes did. (PHI.106). In this latter instance the apparent degenerative shift to the left was not a bad prognostic sign.

Monocytes were raised in seven instances and absolute eosinopenia was also seen on seven occasions. Female neutrophils were absent in three out of six cases.

CHRONIC PYOMETRA Dow type IV.

A total of thirty-two cases were seen of which five were closed and twenty-seven open. The average age was 9½ years and the range 4-13 years.

In these cases again the cervix was liable to open or close at intervals. To enable the reader to correlate cervix changes with the blood picture the clinical histories for each case will be given. The full blood results are in the Appendix, page 67.

CLOSED CASES - Dow type IV.B.

	Date of last oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.26	1 year ago	Moribund. Ill one week. Vomiting dehydrated; died two hours after admission	Dow type IVB. 600 cc. of pus Generalised hormone imbalance at post-mortem.	Coliforms
PHI.42	Unknown	Thin, excess thirst. Anorexia. Ill for one month. Vomiting	Large. 900 cc. of pus. Both horns completely necrotic	Coliforms

Code No.	Date of last oestrus	Clinical signs	Uterus	Bacteriological Examination
PHI.55	Two months ago Not a proper season.	Mammary tumours. Ill ten days. Anorexia. Vomiting.	Large. 350 cc. of pus Endometrium abscessed and slightly necrotic	Haemolytic coliforms
PHI.97	One year ago.	Ill ten days. Thirst. Anoerxia.	-	-
PHI.97a	-	Copious discharge for twelve hours	Small. 5 cc. of pus. Endometrium of left horn more eroded than right	Staphylococci.
PHI.109	Six months ago.	Ill ten days. Anorexia. Vomiting.	-	-
PHI.109a	-	Very ill. Vomiting	400 cc. of pus. Uterus inflamed and endometrium necrotic.	-
	Died during	ovariohysterectomy		

CLOSED CASES

The average results are shown in the following table.

CHRONIC PYOMETRA Dow type IV.

Average values and ranges of variation

Closed Cases

		Average	Range of Variation
R.B.C.	millions per cu.m.m.	4.670	3.395 - 6.100
P.C.V.	%	33.9	26 - 45
Hb.	grms/100 mls.	13.3	10.0 - 16.3
Sed. Rate	m.m. per hour	37.5	5 - 67
W.B.C.	per cu.m.m.	57457	29800 - 115500
Myelo.	per cu.m.m.	143	0 - 1004
Meta.	per cu.m.m.	11128	3338 - 28608
Band	per cu.m.m.	9899	4221 - 18658
Seg.	per cu.m.m.	30375	13600 - 57276
Lymph.	per cu.m.m.	3218	1848 - 5775
Mono.	per cu.m.m.	1950	888 - 6352
Eosin.	per cu.m.m.	170	0 - 577
Misc.	per cu.m.m.	573	0 - 2310

Two bitches showed evidence of anaemia, PHI.55 and PHI.97 and one dehydration, PHI.26.

The sedimentation rates were raised in all cases and varied from 5 - 67 m.m. in one hour. The highest rates were seen in the anaemic cases which is to be expected.

The average total white cell count was higher than in the acute cases, being 57457 per cu.m.m. with a range of 29,800 to 115,500 per cu.m.m. Very high counts (over 60,000 per cu.m.m.) appeared to be a bad prognostic sign as two of the three cases died.

The degree of white cell increase was not associated with the length of illness. PHI.26 had been ill one week and had a count of 115,500 per cu.m.m. while PHI.42 had a count of 33,600 per cu.m.m. and had been ill for one month. In cases where the condition had been present clinically for a short time the white counts were increased when a second sample was taken (PHI.97 and PHI.109). This increase can be very rapid.

A considerable shift to the left occurred in all cases and was usually more marked in cases with high white counts. Case PHI.97 is an exception.

Raised monocyte counts were seen in four cases and absolute eosinopenia in three cases. Female neutrophils were reduced in all cases. The type of bacteria in the uterus did not influence the blood picture.

OPEN CASES Dow type IV.A.

The full results are given in the Appendix pages 68 and 68a.

Code No.	Date of last oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.8	4 months ago.	Intermittent bloody vaginal discharge. Dull, respirations accelerated. Ill five days. Mammary tumours.	Small, full of clotted blood and pus (100 cc.) Endometrium necrotic.	Clostridium welchii.
PHI.8a	-	Recovering from operation four days ago. Had blood transfusion.		
PHI.8a	-	Fully recovered		
PHI.9	A year ago	Vaginal discharge for four days. increased thirst for two months. Eating and not very ill.	Small. 50 cc. of pus. little necrosis but abscesses present in endometrium.	Proteus
PHI.9a	-	Making recovery after operation (two days before). Blood transfusions given.	-	-
PHI.18	Three weeks ago	Mammary tumour. Ill during last season but cleared up. Slight discharge since oestrus. Anorexia, thirst, very sick.	Large. 950 cc. of pus. Some necrosis of endometrium and uterus appeared to be on the point of rupturing in two places.	Coliforms
PHI.18a	-	Eleven days post operative. Recovering well.	-	-
PHI.19	Three weeks	Profuse red discharge for three weeks (since oestrus), otherwise normal.	Small. 40 cc. of pus. Endometrium ulcerated.	Coliforms

Code No.	Date of last Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.28	Two weeks ago.	Ill three weeks. Profuse discharge for three weeks. Thirst; eating.	Small. Right horn bigger than left. 70 cc. of pus right horn necrotic, left horn not necrotic. Bilateral pyometra	E. coli.
PHI.29	Three months ago	Very ill for three days. Thirst, vomiting, vaginal discharge. Blood urea 350 mgs/100 mls.	Small. 10 cc. of pus. Endometrium completely necrotic.	Coliforms and alpha haemolytic strepto-cocci.
		Died day after ovariohysterectomy		
PHI.44	One month ago	Ill for one week. Discharge for one week. Thirst, anorexia. Had similar condition one year ago but this cleared up.	Small, little pus, haemorr-hagic and little necrosis.	Coliforms
PHI.47	Two months ago	Ill and with bloody discharge. for one month. Excess thirst. Dull.	Moderate. 250 cc. of blood (clotted mainly) Endometrium slightly necrotic	Coliforms and Strepto-cocci.
PHI.49 (W.4)	Six weeks ago	Ill six days. Discharge for three days. Excess thirst. Anorexia. Vomiting	Moderate 600 cc. of pus. Endometrium completely necrotic.	Coliforms
PHI.52	Unknown	Ill ten days. Discharge for six days. Anorexia. Thirst and vomiting occasionally	Moderate. 320 cc. of pus. Endometrium slightly necrotic.	Coliforms Proteus

Code No.	Date of last oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.58	One month ago	Dull. Vomiting for one week. Profuse vaginal discharge	Moderate. 80 cc. of pus. Endometrium sloughed	Coliforms
PHI.59	Five months ago.	Ill and discharging for six days. Thirst, vomiting. Anorexia. Dull.	Moderate. 350 cc. of pus. Right horn endometrium more necrotic than left	Klebsiella species
PHI.60	Discharge three months ago.	Anorexia and discharge for two days.	-	-
PHI.60a	-	Treated with antibiotics. No change except for dehydration.	-	-
PHI.60b	-	No change. Discharge less.	Large. 820 cc. of pus. Endometrium completely necrotic.	Alkaligenes spp. and Staphylo-:coccus citreus
PHI.61 almost closed	Six weeks ago.	Mammary tumour. Has had eight litters. Ill six days. Slight discharge for five days. Dull. Anorexia. Thirst	Large. 1000 cc. of pus. Endometrium necrotic in patches	Coliforms
PHI.63	Two months ago (first season for two years)	Ill and bloody discharge for one week. Anorexia Dull.	Small. 30 cc. of pus. Endometrium thick and only slightly necrotic	Coliforms

Code No.	Date of last oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.65	Six months ago	Mammary tumour. Ill six days. Discharge for ten days. Dull. Anorexia. Thirst	Small. 40 cc. of pus. Right horn endometrium more eroded than left	Coliforms Klebsiella spp.
PHI.68	Three weeks ago.	Ill four days. Discharge for one day. Dull. Anorexia. Excess thirst.	Large. 700 cc. of pus. Endometrium necrotic.	Coliforms
PHI.70 (P.41)	Five months ago	Ill ten days. Discharge for two days. Thirst. Started to eat again when discharge started	-	-
PHI.70a	-	No change	Moderate. 160 cc. of pus. Little necrosis of endometrium	Coliforms
PHI.80	Two months ago. One week only.	Ill for one week. Discharge for three days. Anorexia Thirsty. Lively.	Small. 5cc. of pus. Endometrium thick	Coliforms
PHI.84 half closed	Two months ago.	Has had eleven litters. Ill and discharging three days. Anorexia. Vomiting. Excess thirst.	Left horn small. Right horn large contains 150 cc. of pus (not drained). Endometrium necrotic	Staphylo-:cocci and paracolon organisms
PHI.85	Eighteen months ago.	Ill one month ago. Cleared up and recurred three days ago. Profuse discharge for two days. Dull. Anorexia. Dehydrated. Vomiting. Thirsty.	Large. 1000 cc. of pus. Endometrium completely necrotic.	Haemolytic coliforms

Code No.	Date of last Oestrus	Clinical Signs	Uterus	Bacteriological Examination
PHI.93	Nine weeks ago.	Mammary tumour. Ill five days. Discharge for two days (bloody). Anorexia. Thirst.	Small. 10cc. of pus. Endometrium slightly necrotic	Coliforms
PHI.102	Unknown	Ill a week or more. Discharge for three days. Excess thirst otherwise normal.	Moderate. 160 cc. of pus. Endometrium completely necrotic	Coliforms
PHI.103	Fourteen days ago	Vaginal bleeding since oestrus. Only sign.	-	-
PHI.103a	-	No change	150 cc. of pus. Endometrium necrotic	Coliforms
PHI.111	Two months ago	Ill and discharging for two days. Anorexia. Excess thirst for two weeks	Small. 30 cc. of pus. Endometrium thick	Coliforms
PHI.113	Three months ago.	Mammary tumour. Ill, ten days. Discharge for one week. Anorexia (three days). Excess thirst.	Large. 650 cc. fluid. Endometrium completely necrotic Ovarian tumour	Coliforms
PHI.114	Unknown	Unknown time of illness. Copious vaginal discharge. Thirsty; eating well	Moderate. 25 cc. of pus. Endometrium all sloughed.	Coliforms

OPEN CASES

The average results for the cases just described is given in the following table.

Open Cases. Average results and ranges of variation

	Average	Range of Variation
R.B.C. millions per cu.m.m.	4.754	1.340 - 7.160
P.C.V. %	37.6	14.5 - 52.0
Hb. grms/100 mls.	14.1	4.7 - 20.2
Sed. Rate m.m. per hour	23	1 - 75
W.B.C. per cu.m.m.	31434	5800 - 76600
Myelo. per cu.m.m.	59	0 - 742
Meta. per cu.m.m.	3662	111 - 13861
Band per cu.m.m.	4480	496 - 10762
Seg. per cu.m.m.	18874	4165 - 54371
Lymph. per cu.m.m.	2145	522 - 5952
Mono. per cu.m.m.	1633	174 - 7560
Eosin. per cu.m.m.	388	0 - 3001
Misc. per cu.m.m.	192	0 - 1051

In several of these open pyometras the bitch showed no evidence of discharge until she had been ill for several days. In one case the discharge was so slight that it could almost be classed as a closed case (PHI.61) and in another instance only one horn of the uterus was drained (PHI.84).

Anaemia occurred in thirteen out of the thirty-one samples examined (twelve cases). The degree of anaemia varied considerably. Red cells varied from 4,910,000 - 1,340,000 per cu.m.m.

Of /

Of the seven bitches with red cell counts of less than 3,500,000 per cu.m.m. only three had obviously blood stained discharges. The reduction in red cells in the other four cases appears to have been due to t oxic effects upon the bone marrow.

As in other types of pyometra the sedimentation rates were usually elevated but varied enormously. (1 - 75 m.m. in one hour). TThe highest rates were again seen in anaemic cases. (PHI.8 was an exception). In the fifteen cases with normal red counts the sedimentation rates varied from 2 - 45 m.m. in one hour. (Average 8.3 m.m. per hour).

The average total white cell count at 31,434 per cu.m.m. was lower than in closed cases.

Four cases showed white counts within the normal range and in three of these the shift to the left was only slight. (PHI.60, PHI.70 and PHI.114). In the case of PHI.70 the clinical signshad been apparent for ten days.

In animals which had been ill for seven days or longer the increases in total white count were not associated with the length of illness. The following cases show this clearly.

PHI.47 ill four weeks	-	white count 76,600 per cu.m.m.
PHI.28 ill three weeks	-	white count 30,200 per cu.m.m.
PHI.70 ill ten days	-	white count 11,350 per cu.m.m.
PHI.44 ill seven days	-	white count 37,000 per cu.m.m.
PHI.63 ill seven days	-	white count 33,350 per cu.m.m.

Animals which have only shown clinical signs for a short time can have low white cell counts or raised counts.

PHI.60 ill two days	-	white count 5,800 per cu.m.m.
PHI.29 ill three days	-	white count 42,050 per cu.m.m.

A shift to the left was seen in all cases. The greatest shift to the left was usually seen in cases with total white counts in excess of 40,000 per cu.m.m. (PHI.29, PHI.59, PHI.102, PHI.103a).

MORPHOLOGY OF THE CELLS SEEN IN PYOMETRA CASES

In most instances the cells seen are the same as the ones described in the section dealing with normal dogs. Only cells which were not seen in normal dogs will be recorded here.

Platelets

In three out of thirty samples the platelets showed a high percentage of giant forms, Fig. 26. These are seen in normal dogs but not in such large numbers on a single blood smear.

Neutrophils

Myelocytes

These were seen in eight samples out of sixty-seven. They are large cells with specific neutrophilic granules in the cytoplasm. The nucleus is round or shows only a very slight indentation, Figs. 27 and 28.

Characteristic Stab Neutrophil

Such a cell has been described by three of the authors reviewed. From their description it is a larger cell than normal, has a smooth U-shaped nucleus and often shows toxic granulation. Such cells were seen in ten cases out of fifty-eight and its presence would not seem to be characteristic of pyometra. This type of cell is seen in Fig. 28.

Toxic /

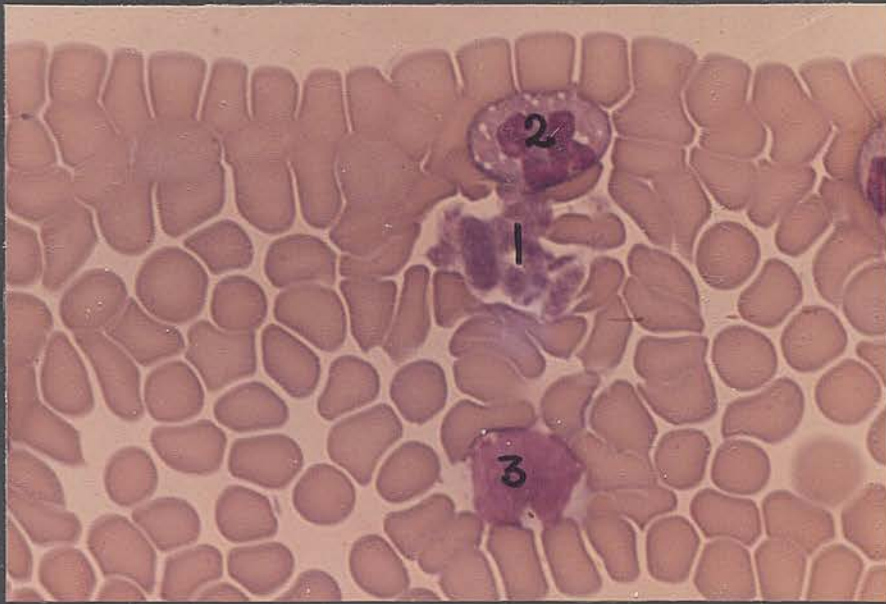


FIG. 26
Case PHI29 Mongrel Female 10 years (Pyometra)
1) group of platelets with giant forms; 2) eosinophil; 3) Type I. monocyte. x 1300

FIG. 27
Case PHI26 Terrier Female 13 years (Pyometra)
1) myelocyte showing granules; 2) metamyelocyte; 3) band form neutrophils; 4) segmented neutrophils; 5) degenerating metamyelocyte. x 1300

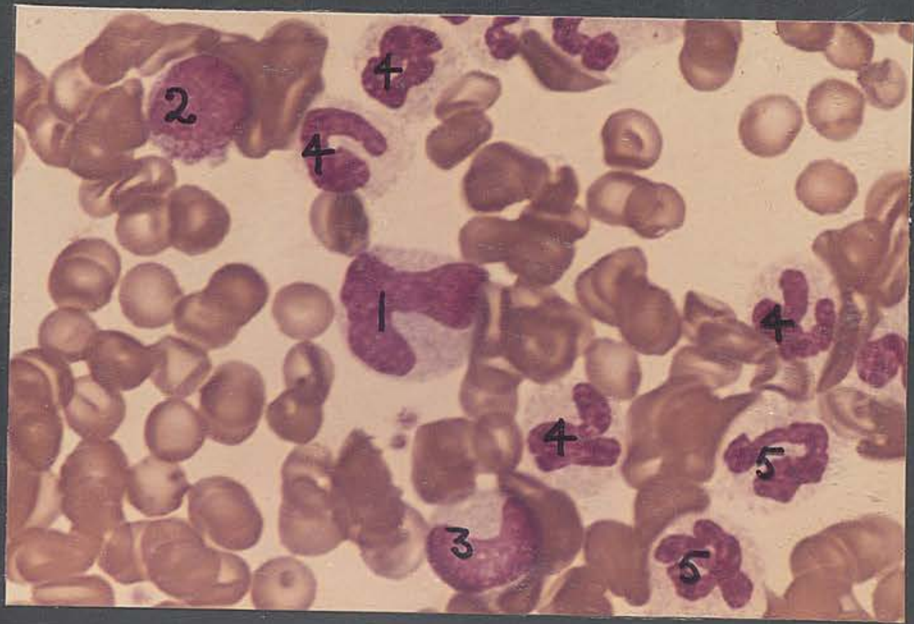


FIG. 28

Case PHI29 Mongrel Female 10 years (Pyometra)
1) "Characteristic" pyometra stab cell; 2) myelocyte; 3) metamyelocyte; 4) band form neutrophil; 5) segmented neutrophil. x 1300

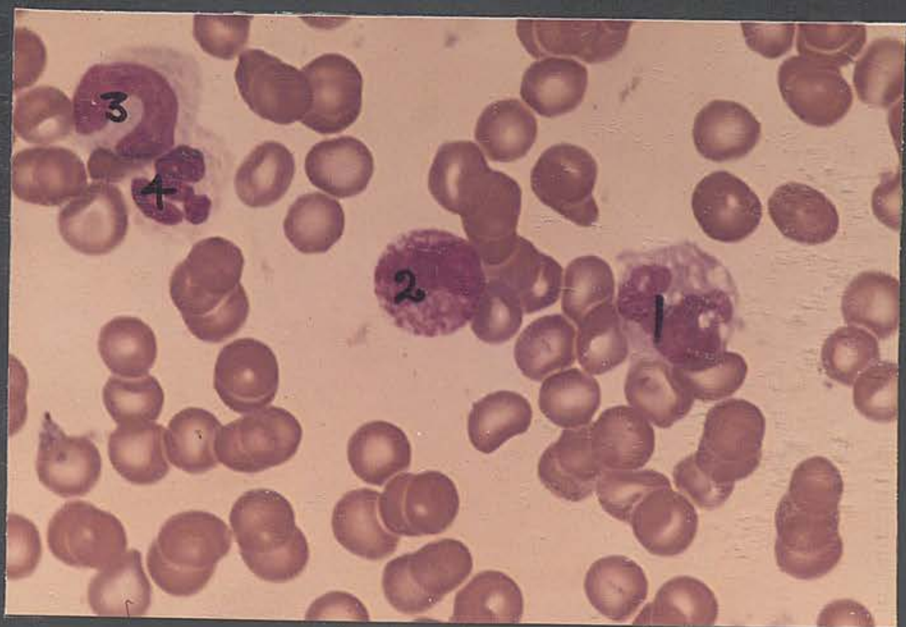


FIG. 29

Case PHI29 Mongrel Female 10 years (Pyometra)
1) Neutrophil showing toxic vacuolation; 2) metamyelocyte with intense granulation; 3) metamyelocyte; 4) segmented neutrophil. x 1300

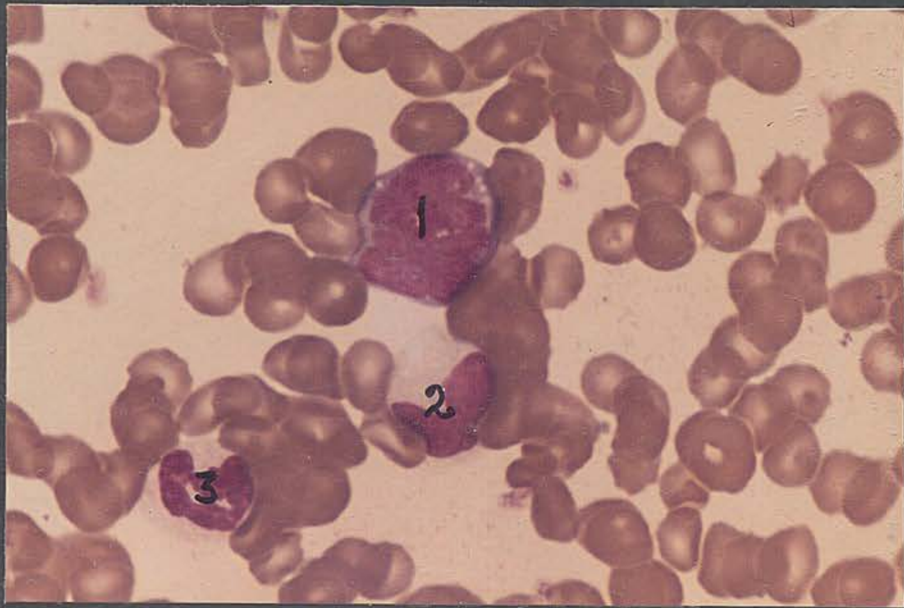


FIG. 30

Case PHI52 Cocker Spaniel Female 10 years (Pyometra)
1) Type I monocyte showing toxic vacuolation; 2) distorted metamyelocyte; 3) segmented neutrophil. x 1300

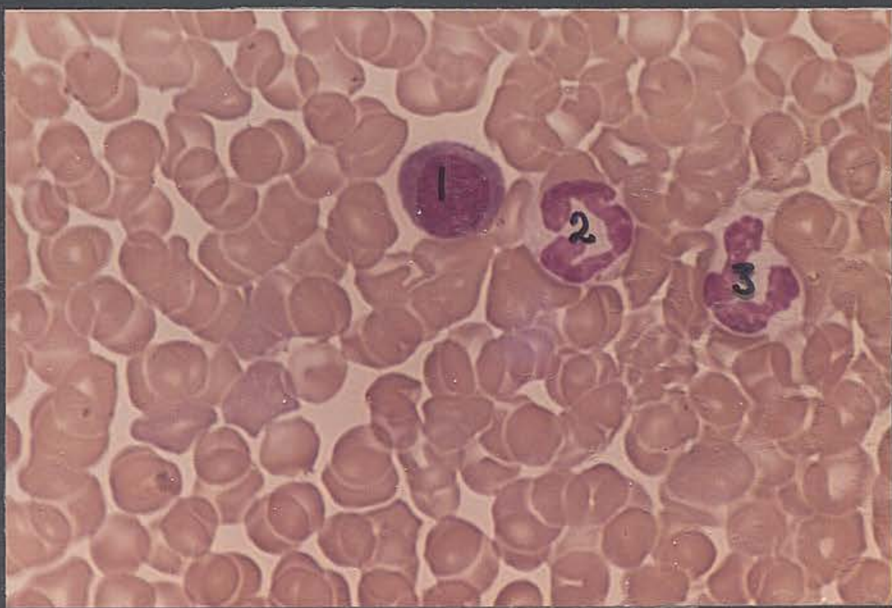


FIG. 31

Case PHI29 Mongrel Female 10 years (Pyometra)
1) Pathological lymphocyte showing basophilic cytoplasm; 2) segmented neutrophil; 3) band form neutrophil. x 1300

Toxic changes in Neutrophils

These were seen in twenty out of fifty-eight samples and are shown in Fig. 29. Toxic changes are seen mainly in dogs with high white cell counts.

Star-shaped Monocytes

These are associated with chronic conditions, especially toxic conditions, and were seen in three out of fifty-eight samples. Monocytes showing vacuolation were also seen in eight samples. (Fig.30).

Toxic vacuolation of Eosinophils

This was seen in three out of fifty-eight samples.

Unidentified Cells

These were especially prominent in cases with high white cell counts and a marked shift to the left. They were seen in twenty out of fifty-eight samples.

Female Neutrophils

The normal average values are 5.4%.

In fifty-seven samples from pyometra cases the average number seen was 0.76 with a range of 0 - 3 per hundred neutrophils. This reduction in numbers is more marked than in pregnancy.

Lymphoblasts were seen in six samples. (Fig. 15).

Lymphocytes

Very darkly staining lymphocytes were seen in a number of pyometra cases. (Fig. 31).

CASES DIAGNOSED CLINICALLY AS PYOMETRA

A certain number of blood samples were taken from bitches which had been tentatively diagnosed on clinical examination as cases of pyometra. In all cases the diagnosis was /

was eventually proved to be incorrect. The blood picture obtained from some of these cases resembled that seen in pyometra and the following results are given to show that the blood picture cannot be considered as truly diagnostic of pyometra. A total of seventeen cases are included here - the details for each individual dog are given in the Appendix, page 69.

The details concerning the clinical signs presented by each dog are given in the following table together with the final correct diagnosis.

The blood pictures for each case are in the Appendix page 70.

Code No.	Date of last oestrus	Clinical Signs	Final Diagnosis
PHI.1	Five months ago	Anorexia. Dull, vomiting, excess thirst. Hormonal alopecia, abdomen enlarged. Ill five days	-
PHI.1a	-	No change	Complete obstruction of bile ducts.
PHI.1b		Now showing signs of jaundice.	
		Dog died two hours after operation to remove bile duct obstruction	
PHI.5	Four months ago	Moribund. Anorexia vomiting. History of excessive thirst.	Diabetes mellitus
		Euthanasia performed and post-mortem.	
PHI.30	Two weeks ago.	Anorexia and excessive thirst. Blood sugar and blood urea normal.	Unknown. Recovered on antibiotic therapy
PHI.38	Unknown	Dirty vaginal discharge. and stiffness of hind limbs. Anorexia.	Calcified intervertebral discs. Vaginal leiomyoma.
PHI.39	One month ago.	Excessive thirst. Anorexia, three days. Enlarged abdomen for three weeks.	Pericarditis Died.

Code No.	Date of last oestrus	Clinical Signs.	Final Diagnosis
PHI.46	Three weeks ago.	Vomiting. Excessive thirst. Lively. Abdomen enlarged.	Diabetes mellitus.
		Euthanasia and post-mortem performed	
PHI.48	Two months ago.	Excess thirst and micturition for one month. Occasional vaginal discharge with blood spots.	Severe Cystitis (diagnosed after laporotomy)
PHI.50	Two months ago.	Dull. Vomiting. Respirations laboured. Thirst. Died	Nephritis. Blood urea 430 mgs/100 mls. Diabetes mellitus Blood sugar. 224mgs./ 100 mls.
PHI.54	Four months ago.	White vaginal discharge. Otherwise normal.	Leiomyoma vagina
PHI.56	Pups five months ago.	Dull. emaciated. Enlarged abdomen. Ill three weeks. Anorexia and excessive thirst. Hard lump in abdomen.	Large ovarian tumour and widespread metastisis.
		Euthanasia after laporotomy	
PHI.66	One month ago	Dirty vaginal discharge for four days. Thin. Anorexia. Excessive thirst.	Multiple large ulcerated vaginal leiomyomata.
PHI.69	Three months ago.	Ill one month. Bloody vaginal discharge for one month. Eating	Foreign body vagina. (4" rigid plastic douching apparatus)

Code No.	Date of last oestrus	Clinical Signs	Final Diagnosis
PHI.72	Three months ago	Vaginal discharge and swollen hind legs	-
PHI.72a	-	No change	Ovariohysterectomy revealed a uterus showing cystic hyper-plastic endometritis. Dow type II.
PHI.72b	-	Developed swollen lymph nodes. Hind legs still swollen	-
PHI.72c	-	All lymph nodes enlarged. Dog well	-
PHI.72d	-	Complete collapse Anorexia. Bloody diarrhoea. Euthanasia	Lymphosarcoma
PHI 77	Unknown	Dirty vaginal discharge and raw bleeding and discharging lesions in mouth and nasal cavity Ill one week	Vitamin B complex deficiency. (Fed on porridge only)
PHI.81	Unknown	Anorexia and excessive thirst.	Unknown. Recovered with antibiotics only.
PHI.86	Not for six years	Vaginal bleeding for two days. Enlarged abdomen	Ulcerated vaginal leiomyoma. Splenic hyperplasia. (Spleen 16 cms. long and 9 cms. wide.)
PHI.86a	-	No change	Sphenic hyperplasia.
PHI.92	Six months ago. Had slight discharge six weeks ago.	Dull. Excess thirst. Eating.	False pregnancy.

RESULTS

Of the seventeen cases examined, eight had blood pictures similar to those seen in Dow type III and IV pyometras. These eight cases came from dogs suffering from the following disease conditions.

Obstruction of the bile ducts	PHI.1
Diabetes mellitus	PHI.46
Cystitis (severe)	PHI.48
Nephritis and diabetes mellitus	PHI.50
Ovarian tumour and metastasis	PHI.56
Lymphosarcoma - terminal stages	PHI.72
Vitamin B complex deficiency	PHI.77
Splenic hyperplasia	PHI.86

Three of these cases showed reduced red cell counts (PHI.48, PHI.72 and PHI.86), and five out of seven cases had increased sedimentation rates.

The total white cell count was increased due to neutrophilia, in all cases, and in seven there was a shift to the left.

None of the eight cases showed the presence of the so called characteristic stab cell of pyometra.

An increased number of monocytes were seen in five cases and absolute eosinopenia in four cases. Complete absence of female neutrophils was seen in four cases.

A case of ovarian tumour and metastasis is described by Owen and Hall (1962) and in their case the blood picture was within normal limits. The metastasis were not as widespread as in the present case and the dog recovered after removal of the tumour.

In /

In cases of vaginal leiomyoma and false pregnancy, the blood picture was unlike that seen in cases of pyometra, and showed no abnormal changes.

DISCUSSION OF BLOOD CHANGES IN PYOMETRA AND SIMILAR CONDITIONS

The earlier workers insisted that the total white cell counts were always elevated and there was a shift to the left in cases of pyometra. This idea is widely held among clinicians to-day. The presence of characteristic cells was also held to be diagnostic.

Lacroix (1947) gives figures for two cases which had total white cell counts within the normal range but showed a shift to the left. No clinical details are given, however.

Dow (1957) showed that normal white cell counts occurred in Type I and II pyometras. His lowest figure for an open type III case was 19,000 per cu.m.m. and for an open type IV case 16,000 per cu.m.m. In closed cases the lowest count was 31,000 per cu.m.m. (type IV).

Irfan (1958) gave figures of 15,000 per cu.m.m. and 18,500 per cu.m.m. as the lowest white cell counts obtained from open and closed cases respectively. In cases with cystic hyperplasia (Dow, type II), the lowest white count was 16,500 per cu.m.m.

Christoph (1961) indicated that 8% of his three hundred and sixty-three cases had total white cell counts of less than 10,000 per cu.m.m. and of these only 1% had a shift to the left. He does not state the types of pyometra involved and they may have been cases of cystic hyperplasia.

Ewald /

Ewald (1961) had twenty cases out of one hundred and fifteen in which the white counts were less than 20,000 per cu.m.m. (All were open cases).

The lowest white cell counts seen by Schalm (1961) in open pyometra cases was 14,000 per cu.m.m. without any shift to the left.

In the present study the case of stilboestrol induced pyometra, and the three cases with uterine changes resembling nymphomania (Dow, type II) showed clinical symptoms but the blood pictures are indistinguishable from those of normal dogs. The presence of bacteria in the uterine contents did not affect the blood picture. These results agree with those of Dow (1957).

In the acute cases of pyometra (Dow, type III) blood picture changes did occur. In both open and closed cases the red cell counts were mostly normal but occasionally fell a little below normal.

Sedimentation rates were raised in both types of acute pyometra, but tended to be elevated more in open cases. No correlation between sedimentation rate and clinical signs could be found, and very considerable variation between one dog and another were seen.

As was found by most of the authors reviewed, the average total white cell counts were higher in cases of closed pyometra than in open cases. In one closed case the count was 11,150 per cu.m.m. and /

and had actually fallen over the preceding five days, although the clinical signs were marked. There was a shift to the left in this case. The monocyte counts tended to be high and the eosinophil counts low in acute closed pyometras.

In acute open pyometra, four of the thirteen samples examined had white counts within the normal range and one of these showed only a slight shift to the left. In two of these four cases the animal had only been ill for a short time. On a second examination of the four animals the white cell counts had risen, in one case very markedly.

No correlation between the white cell count, the clinical signs and the length of illness could be found in acute open cases. In four animals which had been clinically ill for at least seven days and had similar clinical signs, the total white counts varied from 7,100 - 24,125 per cu.m.m. In early cases the total white cell counts often rose sharply but in cases which had been ill for over ten days the counts often fluctuated and some never got above 30,000 per cu.m.m. (PHI.22). As in closed cases the monocytes were often raised and the eosinophil counts depressed.

With chronic pyometras (Dow, type IV) a similar picture was seen. The average white counts were higher in closed cases than in open cases. The average counts for both types of chronic pyometra were higher than those for acute pyometra. The lowest white /

white count in a closed case was 29,800 per cu.m.m. and a shift to the left was seen in all cases. This result agrees with the results of the authors reviewed.

Of the twenty-seven open type IV cases examined, four had white counts within the normal range and of these only one had evidence of a marked shift to the left. In the case of PHI.60 the bitch had only been ill a short time and was given antibiotics which probably helped control infection and to keep the white cell counts low. PHI.70 on the other hand, had been ill for ten days, had only been showing a vaginal discharge for two days, and yet had a white count of 11,350 per cu.m.m. and no marked shift to the left. The case of bitch PHI.85 is even more surprising. Although she showed a moderate shift to the left her white count was only 12,425 per cu.m.m. and yet she had been ill for probably a month, and when her uterus was removed it contained 1,000 c.c. of pus. This bitch made an uneventful recovery after the operation.

In closed cases no correlation between white cell counts, clinical signs and duration of illness could be seen. In eleven bitches showing similar clinical signs and who had been ill for at least seven days, the white counts varied from 11,350 to 76,600 per cu.m.m.

In closed, type IV, pyometra, the rise in monocytes and depression of eosinophils was seen, but this effect was not so obvious in open cases. Both types showed elevated sedimentation rates and these were higher than for type III cases, probably because the number of cases showing low red cell counts was considerable. Elevated sedimentation rates accompany low red cell counts in diseases other than pyometra.

A /

A total of thirteen different clinical conditions have been mistaken for pyometra on clinical examination only, and of these, eight had blood pictures similar to those seen in cases of pyometra. This shows that the blood picture, although a good guide, cannot be relied upon absolutely to differentiate pyometra from other similar clinical conditions.

CONCLUSIONS

1) Dow type II pyometras may show clinical signs but the blood picture is normal.

2) The red cell counts are often within the normal range in cases of type III and type IV pyometra. A low red cell count is seen more often in chronic pyometra. Dehydration will mask anaemia.

3) The total white cell counts are higher in closed pyometra than in open pyometra.

4) The total white cell counts are higher in chronic pyometra than in acute pyometra.

5) A shift to the left and neutrophilia is associated with both classes of pyometra.

6) Normal white cell counts can occur in both chronic and acute pyometra, especially in open cases. In these cases a second examination should be done as the white counts can alter very rapidly.

7) Animals which have been ill for a long time do not necessarily have greatly elevated white counts.

8) /

8) Monocytes are often raised and eosinopenia occurs especially in acute cases. This is associated with severe toxic conditions and not with pyometra alone.

9) Sedimentation rates are raised in both chronic and acute cases and vary considerably from one bitch to another.

10) The number of female neutrophils is depressed in both chronic and acute cases.

11) Toxic vacuolation and degeneration of neutrophils is often seen in pyometra.

12) The type of bacteria isolated from the uterine contents have no bearing on the blood pictures.

13) The total white cell count and the differential count must both be taken into account when attempting to diagnose pyometra from a blood picture. A total white cell count by itself is not sufficient.

MAMMARY TUMOURS

REVIEW OF THE LITERATURE

The literature on mammary tumours themselves and their distribution is extensive, but literature dealing with the changes seen in the blood picture when mammary tumours are present is rare.

Cotchin (1954) and (1956) gives a comprehensive survey on the sites, numbers and types of mammary tumours in the dog. Similar work, but not so extensive, is given by Florey (1962).

Irfan (1958) examined the blood picture in thirty-two cases of mammary tumours. No attempt was made to correlate results with the clinical condition of the animal and the tumours were unidentified. He found 75% of cases to be anaemic. Three cases showed leucopenia, five cases leucocytosis (19,100 - 36,300 per cu.m.m.) and the remainder had normal white cell counts. Twenty cases showed an increased sedimentation rate, the highest being 70 m.m. per hour.

The ranges of variation are as follows:

R.B.C. millions per cu.m.m.	2.9 - 6.38
P.C.V. %	24 - 61
Hb. gms/100 mls.	8.7 - 20.4
W.B.C. per cu.m.m.	5,200 - 36,300

No other account of the blood changes has been found.

PRESENT /

PRESENT WORK

All the animals used in this work were obtained from the Small Animal Clinic and hadbeen brought there because they had mammary tumours and for no other reason. If the animal had some other concurrent disease then it was not used in this survey.

The mammary tumours were removed surgically and then identified. The identification was carried out by Mr. K.W. Head of the Pathology Department.

The clinical condition of each animal has been noted and the cases have been divided into two sections - benign and malignant tumours. Each of these sections has been further sub-divided to divide neoplasms showing necrosis from those showing no evidence of necrosis. A total of thirty-three cases (thirty-seven samples) have been examined and of these nineteen were benign and fourteen malignant. The details for each animal are given in the Appendix, pages 71 and 71a.

RESULTS AND DISCUSSION

Benign Tumours

The clinical history of each dog is given below with the type of neoplasm, its size and the number present. The blood pictures corresponding to each case can be found in the Appendix, page 72.

The column headed clinical signs will only be filled in if clinical signs other than the presence of mammary tumours have been noticed.

MAMMARY TUMOURS - Benign

Code No.	Duration and Signs of Tumour(s)	Rate of Growth.	Size and Number Present	Type of Tumour
PHI.2	Unknown	Slow	1 - 5 x 4 cms.	Cyst adenoma
PHI.7	2 years ulceration of some tumours	Rapid in last few months	7. vary from $\frac{1}{4}$" to $2\frac{1}{2}$" in diameter	Fibro-adenomata and cyst adenomata
PHI.13	8 weeks Bitch 4 weeks pregnant	Rapid since onset of pregnancy	1 - 1" diameter	Fibro-adenoma
PHI.16	9 months -	Slow	4. vary from $\frac{1}{8}$" to 1" in diameter	Adenoma and fibro-adenomata
PHI.24	1 year -	Slow	3. $\frac{1}{4}$" to $\frac{1}{2}$" diameter	
PHI.31	Unknown Off colour two days. Normal when second sample taken	Slow	1. 1" diameter	Mast cell tumour type I.
PHI.64	7 months	Rapid in last 6 weeks	1. $2\frac{1}{2}$" x $1\frac{1}{2}$" x 2.	Fibro-adenoma
PHI.67	Unknown (a long time)	Slow	1. 3" x $1\frac{1}{2}$" x $1\frac{1}{2}$"	Fibroadenoma
PHI.74	3 months -	Quite rapidly	3. vary from $\frac{1}{2}$"-1" in diameter	Fibroadenoma Duct papilloma
PHI.78	First one seen $2\frac{1}{2}$ years ago	All slow	8. Vary from $\frac{1}{4}$"-1" in diameter	Fibro-papilloma
PHI.82	8 weeks. Tumour removed 1 year ago	Rapid	1. 2" x 1" x $1\frac{1}{2}$"	Adenoma

MAMMARY TUMOURS - Benign (Contd.)

Code No.	Duration and Signs of Tumour(s)	Rate of Growth	Size and Number Present	Type of Tumour
PHI.83	2 years	Rapidly over last 6 months	8. vary from $\frac{1}{4}$"-2" in diameter	Fibro-adenomata
PHI.87	18 months	Rapidly in last 3 months	1. 3"x3" x 2"	Fibro-adenoma
PHI.88	3 months	Rapid	1. 2" x 2" x 1"	Fibro-adenoma
PHI.89	3 months	Rapid	1. 2" x 2" x 1$\frac{1}{2}$"	Excessive hyper-plasia and cystic milk retention
PHI.90	Unknown	Unknown	1. 2" x 1$\frac{1}{2}$"x 1$\frac{1}{2}$"	Adenoma producing mucin
PHI.95	4 years	Slow	1. 2" x 1$\frac{1}{2}$" x 1"	Mast cell tumour
PHI.96	18 months	Rapid in last four weeks	1. 3" x 2" x 2"	Fibro-adenoma with bone formation
PHI.101	4 months	Rapid	1. 2"x 1$\frac{1}{2}$"x 1$\frac{1}{2}$"	Adeno-fibroma

The blood pictures in cases of **benign** mammary tumours are often normal.

The red cell counts were low and outwith the normal range in only four samples out of twenty-one examined. In all but one of these cases the tumours were growing rapidly.

Sedimentation rates were increased in eleven out of twenty-one samples, and varied from 2 - 43 m.m. in one hour. Only two cases had sedimentation rates exceeding 10 m.m. in one hour. One of these was pregnant, so the result can be discounted, and the other had a low red cell count and the sedimentation rates may be associated with this.

Platelet counts were high in some cases but within the normal range.

Total white cell counts were normal in all but two cases, which exceeded 15,000 per cu.m.m. In one of these the count returned to normal without any treatment and the rise is probably not associated with the mammary tumour. No definite alterations were seen in the differential white cell counts.

Only one case showed ulceration of the tumour, and the blood picture was normal in all respects.

MAMMARY TUMOURS - MALIGNANT

Code No.	Duration and Signs of Tumour(s)	Rate of Growth	Size and Number Present	Type of Tumour
PHI.27	1 year	Rapid in last few weeks	1. 2"x 2"x 2"	Fibro-adenoma Fibrous sarcoma Lymph node normal
PHI.34	Several months	Rapid	3. ½" x 1" in diameter	Probably mast cell tumour invading the axilla
	Euthanesia due to metastasis two months later			
PHI.37	6 weeks	Very rapid	1. 4½"x 2"x 2"	Adenocarcinoma Lymph node involved
	Ehthanasia metastasis in lungs			
PHI.45	2 months Slight loss of weight	Very rapid.	1. large mass involving four posterior glands. 3½" x 2" x 2"	Adenocarcinoma Lymph nodes involved
	Later history unknown			
PHI.51	Unknown. Noticed one week ago	Unknown	1. 3" x 1½"x 1½"	Adenoma Adenocarcinoma Lymph node normal
PHI.53	Several months Ulcerated	Rapid in last four weeks	1. 4"x3½"x 3"	Fibro-adenoma Osteo-sarcoma
	Euthanasia 2 months later by another veterinary surgeon			
PHI.73	6 months Tumour ulcerated	Rapidly in last 8 weeks	1. 6" x 3"x 3"	Fibro-adenoma to adenocarcinoma and fibrosarcoma Lymph node involved
	History afterwards unknown			
PHI.75	18 months	Slow	4. largest 2" and smallest ½" in diameter	Spheroidal cell carcinoma
PHI.75a	-	-	-	
PHI.75b	Re-occurred Losing weight	Very rapid	1. 1½"x2" x 2"	Adenocarcinoma with metastases in kidney and lungs.
	Ehthanasia and Post-mortem			
PHI.76	8 months	Rapid in last two months	1. 2" x 2" x 1"	Papilloma and adenocarcinoma Lymph node normal

MAMMARY TUMOURS - Malignant (Contd.)

Code No.	Duration and Signs of Tumour(s)	Rate of Growth	Size and Number Present	Type of Tumour.
PHI.91	Unknown	Unknown	1. $2\frac{1}{2}$" x 2" x 2"	Adenocarcinoma Lymph node involved
	Later history unknown			
PHI.94	3 months Ill. Tumour makes walking difficult Tumour necrotic.	Very rapid	1. 5"x5"x4"	Fibroadenoma-sarcoma (anaplastic)
	Died after removal of tumour			
PHI.99	6 weeks	Rapid	1. 3"x$1\frac{1}{2}$"x1"	Adenocarcinoma Lymph nodes involved
	Metastases. Euthanasia 3 months later			
PHI.100	3 weeks	Very rapid	2. 1-2" long Runs into axilla Weight 1 lb.	Adenocarcinoma Lymph node involved.
	Metastases in lungs. Euthanasia			
PHI.112	3 months Ill. Hardly able to walk. Tumour necrotic.	Very rapid	1. 6" diameter Weight 3 lbs.	Necrotic adenocarcinoma Metastases in lungs
	Euthanasia and post-mortem			

The blood picture in cases of malignant tumours showed more change than was the case with benign tumours.

Four cases showed severe necrosis or ulceration of the tumour and these cases will be discussed separately. They are PHI.53, PHI.73, PHI.94 and PHI.112. (See Fig. 32).

Tumours without necrosis

Twelve samples from nine dogs were examined. Of these only two dogs had reduced red cell counts. One of these was later shown to have widespread metastases, the other recovered.

Six dogs had sedimentation rates of 5 m.m. in one hour or over. Two of these dogs recovered, two were known to have had metastases and two probably had metastases, as the drainage lymph node was involved. Of three dogs with sedimentation rates of less than 3 m.m. in one hour two had metastases which eventually killed them.

Platelet counts were normal in all cases.

The white cell counts of all dogs but one, were within the normal range. This case (PHI.75b) had widespread metastases and a total count of 17,450 per cu.m.m. and a shift to the left. One other dog showed a slight left shift.

Female neutrophils were not depressed as in pyometra.

Tumours with Necrosis or Ulceration

Two cases showing necrosis and two showing ulceration occurred. One case with ulceration had a low red cell count but the other was normal. Both dogs with necrotic tumours had low red cell counts.

Sedimentation /

Sedimentation rates were increased, but only slightly in one case with necrosis. Platelet counts were normal.

Both ulcerated cases had white counts within the normal limits and no shift to the left. The two cases with necrosis had raised white counts 25,900 - 57,350 per cu.m.m. and a marked shift to the left. They both showed absolute eosinopenia.

CONCLUSIONS

Benign and malignant mammary tumours, without necrosis, cause little change in the blood picture. The most consistent change is an elevated sedimentation rate but this does not always occur. Red cell counts may be reduced but not usually sufficiently to cause clinical signs of anaemia.

The white cell counts are within the normal range and no shift to the left is seen even in cases with very malignant tumours which have started to metastasise.

Tumours showing evidence of widespread necrosis (usually malignant) cause elevation of the white cell counts and a shift to the left. The sedimentation rates are increased and red cell counts low. These changes are due to the necrosis and not directly to the tumour.

From these observations it is possible to suggest that a dog with mammary tumours which shows no evidence of necrosis and has an elevated white cell count, has either some other pathological condition present, or widespread metastases.

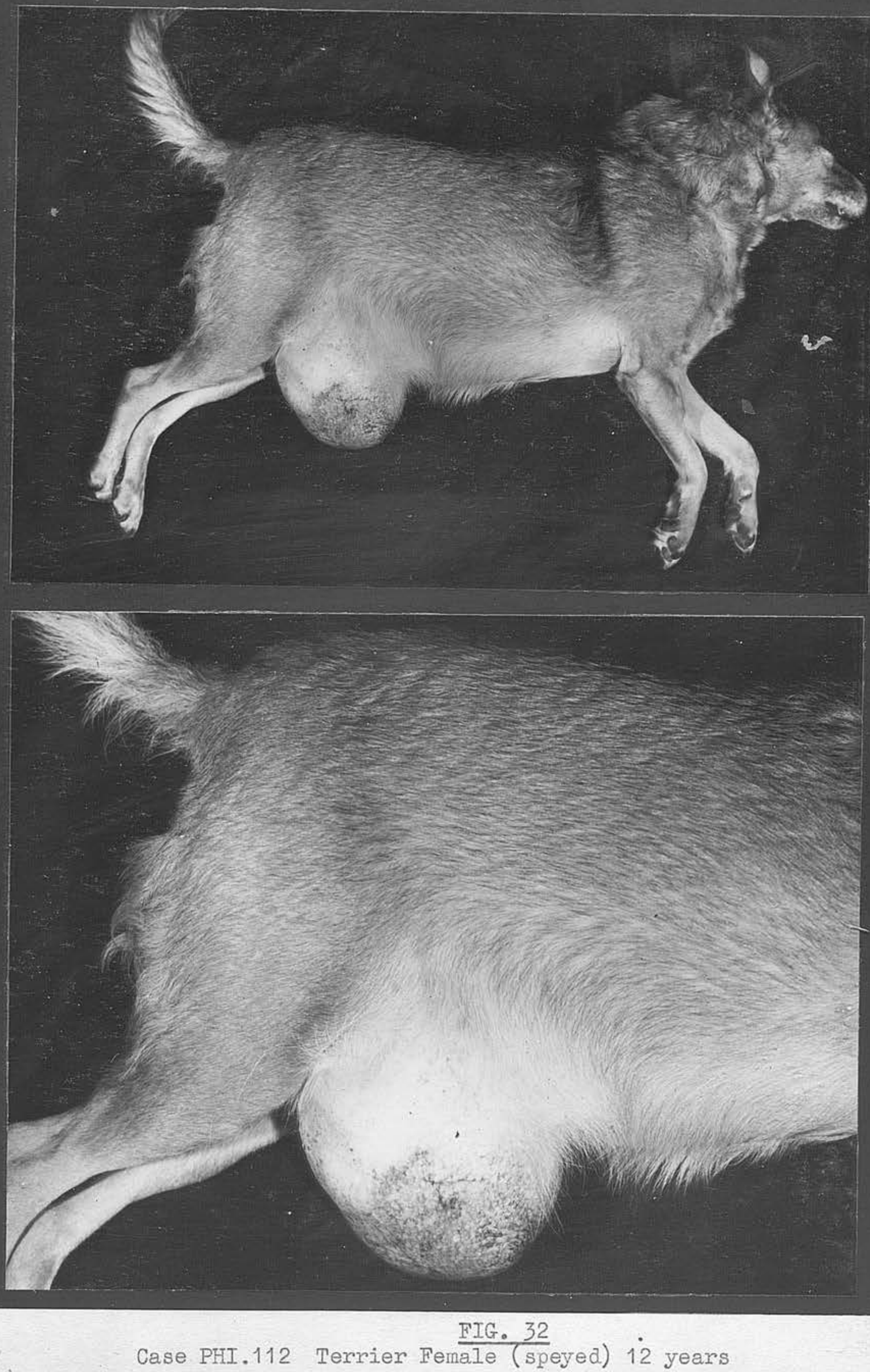


FIG. 32

Case PHI.112 Terrier Female (speyed) 12 years

Large mammary tumour. This was an Adenocarcinoma. When the tumour was opened severe necrosis was seen, although the skin showed no evidence of ulceration.

DISCUSSION OF THE RESULTS OBTAINED FROM NORMAL AND ABNORMAL DOGS

A section discussing the results has been given with each part of this work and this final discussion aims at correlating all the various results together.

As has been stated already, the variations given by some authors on the normal dog's blood values show very wide variations indeed, and if these figures are used as a guide many pathological cases would have apparently normal values. The best examples of this type of result are the following:-

Variations in red cell counts

2.502 - 8.126 millions per cu.m.m.	Sherrington 1894
4.2 - 8.03 millions per cu.m.m.	Busch and Van Bergen 1902
4.00 - 8.00 millions per cu.m.m.	Burnett 1917
2.44 -11.016 millions per cu.m.m.	Mundhenk 1941
3.28 - 8.70 millions per cu.m.m.	El Hindaway 1948

The results of Mundhenk show particularly large variations.

In a similar way the total white cell counts for so-called normal dogs have been shown by some authors to have enormous variations. Examples of this are as follows:-

11,000 - 28,000 per cu.m.m.	Dawson 1900
6,000 - 31,000 per cu.m.m.	Burnett 1917
5,650 - 19,200 per cu.m.m.	Mayerson 1930
2,700 - 21,000 per cu.m.m.	Mundhenk 1941
7,900 - 18,400 per cu.m.m.	Mulligan 1941
545 - 27,950 per cu.m.m.	Rekers and Coulter 1948
6,000 - 18,000 per cu.m.m.	Kirk 1964

The last result is taken from the Appendix of a very recent publication indicating that figures showing a wide range of variation are still being widely used as a guide to normal values.

Many /

Many of the other authors quoted in the literature review mention figures with a narrower range of variation.

The figures just given show what appears to be an absurd range of variation in several cases. However, the workers who produced these results must have known what a healthy normal dog looked like and presumably only gave results from this type of dog. (A few exceptions have been mentioned in the review of the literature). This, of course, leads one to think that these ranges may be correct, and it has been the purpose of this work to find out if such large variations can occur in normal dogs, and if so, why.

The work already presented shows clearly that these wide variations do occur but that several modifying factors have to be introduced to get the picture into its proper perspective. The total white cell counts seen in the course of this work varied from 4,150 per cu.m.m. to 23,950 per cu.m.m. and if the definition of normality was given as a clinically normal dog (including pregnancy) the upper end of this range can be extended to 56,325 per cu.m.m. From these figures it may be stated that the range of a dog's normal white cell count is 4,000 to 24,000 per cu.m.m. but this is not in fact the case, even though the figures were obtained from normal dogs. In short /

short, the normal blood values for a dog vary considerably, but the range depends on the influence exerted by factors such as environment and age.

Many of the factors influencing the blood picture have been investigated in this work, and some, but not all, have been shown to cause appreciable variations.

The first section dealt with showed that the blood picture of an individual animal varies from hour to hour, from day to day, and from week to week. In each instance the values fluctuated up and down, rather than showing a continuous trend in one direction. The results indicated that the variation from one day to the next are greater than the variations from one week to another but this is a false impression. The extent of the variations depends greatly on the environmental influences. Two of the dogs used to assess daily variations came from Kennel S where considerable environmental influences are found, and another was P.77, a dog which showed individual variations unlike any of the other dogs examined. The dogs used to assess weekly variations came from Kennel H and the effect of environmental influences is less here so that the variations between one sample and the next are not so large. This type of variation is never big enough to explain the wide variations in red or white cell counts shown by the authors mentioned.

Sex /

Sex has no appreciable effect on the blood picture, but age, on the other hand, causes considerable differences.

The red cell counts, packed cell volumes, haemoglobins and specific gravities are low at birth and increase gradually to reach adult values when the dog is one year of age. The complete range encountered in this work was from 3,450,000 per cu.m.m. to 8,370,000 per cu.m.m., but this is not the range for a normal dog. This can only be expressed according to the dog's age and gives the following approximate ranges (excluding greyhounds):

Day old puppies	3.5 - 4.0 millions per cu.m.m.
1-3 months	4.52 - 5.59 millions per cu.m.m.
3-6 months	4.85 - 6.66 millions per cu.m.m.
6 months-1 year	5.49 - 7.11 millions per cu.m.m.
Over 1 year	5.50 - 7.83 millions per cu.m.m.

Packed cell volumes, haemoglobins and specific gravities show the same variations.

White cell counts are not influenced as much by age. They tend to be higher in young puppies and fall slowly as the dog ages. These variations do not cover the whole range of counts seen in this work, and factors, other than age, influence the white cell counts more markedly.

Neutrophils and lymphocytes decrease with age, but this decrease is also affected by environmental influences. Platelet counts are highest in very young dogs, breeding females and in pregnancy.

The /

The environment in which the dog lives influences the variations in red cell counts and allied estimations only slightly. Only when the feeding is poor does any marked variation occur. The total white cell counts and differential counts, on the other hand, are affected to a much greater degree. One can go as far as to say that it is environment which has the most marked effects on the blood picture of normal dogs and provides the greatest hazard when analysing the blood pictures as an aid to the diagnosis of disease.

These environmental effects are most marked in large kennels and least marked in pet dogs living in the country. Greyhounds are an exception to this rule. The ranges of white cell counts forthe various types of environment are given in the standard blood picture and the values for dogs over one year of age can be summarised as follows:

Large Kennels	9,000 per cu.m.m. to 21,270 per cu.m.m.
Small Kennels	6,000 per cu.m.m. to 15,500 per cu.m.m.
Pets	5,000 per cu.m.m. to 15,000 per cu.m.m.

The differential white cell counts are influenced in the same way and we find that neutrophil counts are higher in dogs from large kennels than in pets. Eosinophil counts also vary but depend to some extent on the degree of internal parasitism which is usually present to a greater extent in dogs from large kennels than from pet dogs. Lymphocyte counts may rise in association with immune responses.

Breed /

Breed had little or no effect on the blood picture of normal dogs with the exception of greyhounds. In these dogs white counts tend to be low and red counts and allied estimations high. The concentration of these cellular elements is regulated almost entirely by breed in the greyhound.

The effect of work is to raise the red cell counts, packed cell volumes and haemoglobins but these changes are easily masked by environmental effects. Any increase is slight and does not alter the picture noticeably.

Exercise, feeding and travelling influence the blood pictures to a slight degree only and any change is a transient one. These factors do not play an important part in blood picture variations and cannot be ascribed as the reason for the large ranges of variation described by other authors.

Lack of water raised the red cell counts, packed cell volumes, haemoglobins and specific gravities to a considerable extent and the change is permanent as long as the method of watering is abnormal. These changes may in part account for the high figures given by some authors but not in all cases. The highest figure given by Mundhenk (1941) can only be ascribed to haemoconcentration in the pathological sense.

Pregnancy, oestrus periods, and mummification show marked effects on the red cell, white cell and platelet counts, but false pregnancy has no effect at all.

The /

The effects of oestrus periods are slight and mainly concerned with temporary increases in white cell counts and platelet counts. The effects of pregnancy, however, last longer and are considerable. The packed cell volumes and red cell estimations fall steadily throughout pregnancy and can reach values which would be considered pathological in non-pregnant adult dogs, e.g. red cell counts of 3.91 millions per cu.m.m. and packed cell volumes of 33%.

The total white counts and differential counts reach increased values, which could be confused with a pathological abnormality if pregnancy was not suspected.

Foetal mummification of the type described in this thesis is rare, and the results obtained indicate that it would be impossible to distinguish it from pyometra, by means of a blood picture alone.

The conditions of pregnancy and oestrus are usually easily recognisable entities and changes in the blood picture, outwith the normal ranges given in the text-books, should be expected if they are present.

When examining the various cellular constituents of the blood with a view to the diagnosis of a disease process, various factors, which modify the normal values one would expect, have been shown to occur. The main effects can be summarised in the following way and these specific influences shown. Each factor is given in its order of importance.

Factors /

Factors affecting the red cell counts, packed cell volumes, haemoglobin and specific gravities

a) Age - considerable increased in all values from birth to one year.

b) Pregnancy - considerable reduction in all values as pregnancy progresses.

c) Environment - slight effects only. Poor feeding reduces values, lack of water increases them.

d) Breed - concerns greyhounds only. High values.

e) Work - very slight, easily masked increase.

Factors affecting total and differential white cell counts

a) Environment - the most important single factor in non-pregnant dogs.

b) Pregnancy - considerable increased in later stages of of pregnancy.

c) Age - decreased values, especially lymphocytes, but changes can be masked.

d) Oestrus - increased values for a short time only.

e) Breed - Greyhounds only - low values.

Factors affecting platelet counts

a) Pregnancy - considerable increase

b) Environment - increased values in dogs housed in large groups. Possibly associated with wounds.

c) Oestrus - slight transient increases.

d) Age - higher values in young pups only.

The factors mentioned should all be taken into consideration

when /

when assessing what the probable normal blood values are for any individual dog. The other factors which are important when assessing normal values are the methods used for any particular estimations and the individual views of the person doing the estimations. Examples of these factors can be seen in this work. The haemoglobin values are higher than those described by the majority of earlier authors as the method of estimation is different. The number of band form neutrophils is higher as the author classes two types of cell into the one group.

Variation in cell types have been seen in normal dogs. The presence of small numbers of proplasmocytes, lymphoblasts, mural lymphocytes or lymphocytes with bean-shaped nuclei does not indicate any pathological process. The eosinophils of greyhounds are different from those of other dogs.

Having described the various ways in which the normal blood picture can be altered we must now apply these variations to the abnormal dogs which have been studied. All the dogs used in the abnormal section fall into the category of pets or working dogs. They will have blood values falling within the ranges described in the standard blood picture for this class of dog, if they are normal.

In the majority of cases suffering from pyometra this was not the case. The stilboestrol induced pyometra and the Dow (type II) had normal blood pictures, but in most of the acute and chronic cases the blood picture showed distinct abnormalities.

The /

The normal maximum white cell count has been shown to be around 15,000 per cu.m.m. for the pet group. Band forms have a maximum of 1,500 and metamyelocytes 260 per cu.m.m. for adult pet dogs. Of the fourteen samples taken from cases of acute closed pyometra (Dow type III) thirteen had total white counts exceeding the normal maximum. The only case with a normal count had a shift to the left with metamyelocytes and band forms above the normal limits.

Eight out of thirteen samples from open acute cases had white cell counts in excess of 15,000 per cu.m.m. and the remaining five had metamyelocyte counts in excess of 260 per cu. m.m.

From a total of nineteen samples from both open and closed (type III) cases, three showed a decreased red cell count (normal minimum 5,000,000 per cu.m.m.). Seventeen samples out of twenty had sedimentation rates above the adult pet maximum of 1.6 m.m. in one hour.

In the Dow (type IV) pyometras a similar situation was seen. In all closed cases the total white cell counts, metamyelocytes and sedimentation rates were above normal. Out of twenty-seven (type IV) open cases twenty-three had increased total white cell counts and of the remainder only one sample had no detectable shift to the left. Three days later this case had a total white count just in excess of normal values. Sedimentation rates were elevated and only two cases had values which were normal.

It /

It can be definitely concluded therefore that marked changes in total white cell and differental counts are associated with pyometras. Sedimentation rates and sometimes red cell counts are also affected. It is, however, possible to get an early case with a normal blood picture, but this picture should alter a day or two later.

It was shown that several different clinical conditions can give rise to a blood picture which is similar to that seen in pyometra, (types III and IV), and which also gives rise to similar clinical signs. The blood pictures could be confused in that the total white cell counts were above normal and in many cases there was a shift to the left, an increase in sedimentation rate and a decrease in red cell counts.

In the light of these results it is not possible to diagnose pyometra with any degree of certainty from a blood picture alone. A thorough clinical examination ofthe case is essential to obtain a correct diagnosis. If the clinical findings are inconclusive, however, a blood picture is of considerable help in the diagnosis of pyometra, but not a fool-proof aid.

Benign or malignant mammary tumours, showing no evidence of ulceration or necrosis, do not cause any alteration in the blood picture apart from elevation of the sedimentation rates in some cases. Rapidly growing tumours have no more effect on the blood picture than do slow growing tumours.

Necrosis /

Necrosis of a tumour (usually malignant) will cause elevation of the white cell counts and a shift to the left as well as increasing the sedimentation rates. If the tumour has given rise to widespread metastasis the white cell count may also be elevated.

When attempting to diagnose pyometra, mammary tumours, or any pathological condition by means of a blood picture, it is essential to examine all aspects of the picture. A white cell count alone, or a differential white cell count alone, is not sufficient, and, in fact, may lead to erroneous conclusions. In short, such a procedure is a waste of time.

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